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LIST OF AUTHORS

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Plasma Phospholipid Levels and Distribution in Human Maternal Blood and in Blood from the Umbilical Cord

By

Kim Cramér and Olle Vikrot

A pronounced hyperlipemia occurs during pregnancy (1, 3, 13) The cause is not known though many possibilities have been discussed A characteristic finding of this hyperlipemia is a change in the plasma phospholipid composition (13 15), and to explain fully the alterations in lipid metabolism during pregnancy this change in phospholipid pattern must be taken into consideration

The aim of the present investigation was to compare the plasma phospholipids from the mother at delivery with those from cord blood It was considered possible that investigation of cord blood might give some information on the mechanism of the hyperlipemia If the phospholipid patterns were the same in mother and in child a direct transport of phospholipids through the placental barrier could perhaps occur or as another possibility, the factor responsible for the phospholipid alterations in the mother might be able to pass this membrane to a certain extent

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Material and methods

Peripheral venous blood was obtained from 18 women with normal pregnancy shortly before delivery maximum interval about 4 hours The majority of the women had had a snack or light meal within 12 hours before the specimen was taken From the children all of whom were full term blood was taken immediately after the delivery of the placenta The blood was collected in heparinized tubes and centrifuged in the cold and the plasma was immediately extracted with 15 volumes chloroform methanol 1 1 v v The extract was subjected to phase partition dialysis with 0 9 per cent saline solution (11) From these extracts duplicate aliquots were taken for estimation of total lipid soluble phosphorus 12 Aliquots for phospholipid separation were stored with addition of 1 part of methanol

The composition of the phospholipid fraction was determined in duplicate with thin layer chromatography on silica gel according to a previously described method (15) From the extracts of infant blood aliquots containing about 10 micrograms of phosphorus were available from the mothers extracts 15—20 micrograms

Standard statistical methods were used (10) Calculations were performed on the difference between the values for mother and

TABLE I Mean values and standard errors of the means (S E M) for total and individual plasma phospholipids in 18 mothers and children The differences were obtained by comparing the values from each mother with those from her child P refers to the significance of the differences against zero

	Total phospholipids (mM)	Individual phospholipids							
		% of total P lipids				mM			
		PE	Lec	Sph	LL	PE	Lec	Sph	LL
Mothers	3 83	4 8	73 8	19 9	1 5	0 18	2 83	0 76	0 06
S E M	0 111	0 21	0 38	0 40	0 08	0 008	0 090	0 021	0 004
Children	1 08	2 0	67 6	22 3	8 0	0 02	0 73	0 24	0 08
S E M	0 068	0 18	0 40	0 39	0 39	0 002	0 048	0 017	0 005
Difference (mother—child)	2 75	2 8	6 1	2 4	6 5	0 161	2 094	0 518	0 026
S E M	*0 098*	*0 19*	*0 50*	*0 52*	*0 37*	*0 008*	*0 080*	*0 021*	*0 004*
P	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001

PE phosphatidylethanolamine Lec lecithin Sph—sphingomyelin LL=lysolecithin

child The significance of this difference against zero was tested using Student s t test

Results

On thin layer chromatography the ex tracts from the children showed the same four main spots as those from the mothers The mean recovery of phos phorus applied to the plates was 90 per cent for both mothers and children

Table I presents the mean values for mothers and children and the mean differences

The mothers showed the changes typical of pregnancy (13 15) Thus all phospholipids were increased except lysolecithin which was decreased The percentage values of lecithin and phosphatidylethanolamine were increased while sphingomyelin and lysolecithin were decreased

The children differed markedly from their mothers and showed more nearly the same phospholipid pattern as non pregnant women (8), though the lyso lecithin percentage was somewhat higher Total phospholipids were much lower in the children than in the mothers as were absolute values for phosphatidyl ethanolamine lecithin and sphingomyelin However, lysolecithin was found in a higher absolute concentration in the children This was a constant finding, each child had higher lysolecithin level than its mother

All the differences between mothers and children, absolute as well as relative, were significant at the 0 1 per cent level

When correlation coefficients were calculated for each mother and her child, a low grade correlation was found for total phospholipids (r = 0 48), absolute lysolecithin values (r = 0 52) and relative phosphatidylethanolamine values (r = 0 51) These correlation coefficients were significant at the 5 per cent level while other correlations were not significant

Discussion

The phospholipid composition of the mothers plasma fitted well with that expected at term according to regression equations presented in another paper (13) The absolute level for total and individual phospholipids was somewhat lower in the present material One explanation might be that the samples were taken at the end of labor and the level of phospholipids (except lysolecithin) is known to decrease rapidly after delivery (14)

While it is well known that newborn children have much lower total phospholipids than their mother (9) only a few observations have been made on the phospholipid composition of umbilical cord blood Helmy and Hack (7) compared maternal and cord blood by visual comparison of paper chromatograms and considered 'cephalin in cord blood to be in lower concentration than in maternal plasma Gotlieb et al (4), also using paper chromatography found neither phosphatidylethanolamine nor lysolecithin in cord blood, but with their method they could not find these phospholipids in adult blood either

Recently Graven et al (5) investigated several new born children by quantitative paper chromatography and found values for lecithin and sphingomyelin well in agreement with those in the present study Lysolecithin was found in a somewhat higher concentration perhaps because the authors used not plasma but serum which gives higher values than plasma (15) Phosphatidylethanolamine was not measured

From the present data a direct transfer of phospholipids across the placental membrane appears improbable A possibility for the dissimilar composition might, however, be a selective transfer across the placenta of different phospholipids Whether such a transfer of phospholipid occurs in humans is not known The report of Boyd and Wilson (2) suggested that the fetus could take up phospholipids and cholesterol from the placenta However, if this occurs the phospholipids do not necessarily come from the mother but may be synthesized in the placenta Evidence from animal studies reviewed by Hagerman and Villee (6) speaks for synthesis by the fetus itself as the major source of its lipids Our data do not allow any conclusion on this issue

The marked difference in phospholipid composition between mother and child makes it seem less probable that the altered phospholipid pattern in pregnancy hyperlipemia could be due to a factor which can be transferred unaltered across the placenta However, the present data do not prove that such a factor is not the cause There might be a difference in target organs e g the maternal and the fetal liver

Summary

Plasma phospholipids were determined in 18 women during labor and in cord blood after delivery of the placenta

Total phospholipids were higher in the mothers as were the individual phospholipids, except lysolecithin which was at a higher level in cord blood

The significance of these findings is discussed

References

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Lipolysis in Plasma after Subcutaneous, Intramuscular and Intravenous Heparin in Small Doses

By

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During the last decade several workers have investigated the relationship between heparin and the post heparin lipoprotein lipase in blood after various routes of heparin administration Some investigators have used heparin in a rather high dosage Engelberg (6) injected a minimum dose of about 5 000 IU intravenously and used for subcutaneous administration at least 10 000 IU Other investigators have reported lipolytic response after low doses of heparin Sailer et al (13) studied the lipoprotein lipase fifteen minutes after intravenous heparin and found a maximum lipolytic response after a dose of 12 IU per kg body weight (a total dose of about 1 000 IU)

In the present study a low fixed dose of heparin was given and the lipoprotein lipase activity was measured at short intervals for some hours This should contribute to an amplification of our knowledge of the lipolytic response in relation to dose and mode of application Heparin was administered intravenously intramuscularly, and subcutaneously in a dose of 12 IU per kg body weight In all cases the lipolytic activity in plasma without previous heparin was estimated to establish a reliable baseline

The lipoprotein lipase activity was measured by an in vitro assay using the incubation mixture described by Korn et al (10) The enzyme activity was expressed as the amount of non-esterified fatty acids (NEFA) produced during the hydrolysis of the triglyceride moiety under the assay conditions

Materials and methods

Test subjects

Five healthy male students aged 20 to 30 years were admitted to the hospital ward for the preceding 10 hours and kept fasting throughout the test period An interval of at least a week between two tests in the same person was considered necessary

Heparin

For intravenous and subcutaneous use Heparin Leo containing 5 000 IU per ml diluted with distilled water to a concentra

Submitted for publication June 15 1965

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Plasma phospholipids were determined in 18 women during labor and in cord blood after delivery of the placenta

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TABLE I Relation between time and lipoprotein lipase activity The left sub-division in each of the four columns refers to the time in minutes after the beginning of the test period or, where applicable after the administration of heparin The right sub division refers to the enzyme activity (micromoles non esterified fatty acids produced per 60 min per ml plasma)

Spontaneous		Subcutaneous		Intramuscular		Intravenous	
Time	Enzyme activity	Time	Enzyme activity	Time	Enzyme activity	Time	Enzyme activity
Test subject BMK							
0	0 35	0	0 65	0	0 50	0	0 55
60	0 50	10	0 40	5	0 55	1	2 05
120	0 45	30	1 65	24	1 30	6	7 45
240	0 45	60	1 45	60	1 45	15	7 35
360	0 65	120	1 00	95	4 75	45	3 20
420	0 65	270	0 45	130	2 00	65	2 00
		390	0 55	200	0 85	90	1 20
		510	0 60	280	0 50	120	0 80
				380	0 85	155	0 65
				480	0 80	200	0 55
						300	0 55
Test subject KBH							
0	0 60	0	0 50	0	0 65	0	0 55
60	0 55	11	0 45	15	1 60	6	9 95
120	0 55	30	1 00	30	2 00	15	7 90
180	0 55	60	1 05	60	2 65	25	5 50
260	0 50	180	0 70	90	2 70	45	3 15
315	0 50	280	0 70	120	1 60	65	1 45
380	0 60	370	0 70	210	1 00	90	1 00
		460	0 40	290	0 90	120	0 85
		570	0 40	370	1 00	200	0 60
						300	0 55
Test subject KND							
0	0 35	12	0 40	0	0 55	0	0 55
60	0 30	30	0 60	5	0 85	1	1 40
120	0 35	60	0 80	15	1 10	6	8 70
240	0 35	125	1 05	36	1 45	15	7 70
300	0 45	220	0 50	60	1 20	25	5 45
360	0 35	315	0 25	120	1 15	45	2 90
420	0 35	420	0 45	200	0 90	90	0 85
		480	0 25	280	0 45	120	0 85
						150	0 90
						200	0 75
						300	0 65
Test subject LI							
0	0 60	0	0 55	0	0 65	0	0 40
60	0 75	30	0 90	5	0 55	1	4 55

Table I Cont

Spontaneous		Subcutaneous		Intramuscular		Intravenous	
Total	Enzyme activity	Total	Enzyme activity	Total	Enzyme activity	Total	Enzyme activity
Test subject I I							
120	0.50	70	1.40	14	1.20	22	6.50
180	0.55	120	0.75	30	1.30	45	2.80
240	0.50	150	0.65	60	1.20	90	0.85
360	0.55	210	0.85	90	1.15	120	0.75
420	0.50	275	0.74	130	1.00	145	0.70
		360	0.45	200	0.80	210	0.[illegible]5
				305	0.80	300	0.95
				400	0.70		
				500	0.60		
Test subject QM							
0	0.60	0	0.25	0	0.40	0	0.30
60	0.30	11	0.45	5	0.70	1	[illegible].05
120	0.35	30	0.45	15	0.70	6	9.00
180	0.25	60	1.30	37	0.90	15	8.90
360	0.30	120	1.00	60	1.10	25	[illegible].30
420	0.30	210	0.45	90	1.05	45	3.35
		300	0.35	130	0.75	65	1.[illegible]0
		390	0.45	200	0.50	90	0.85
		480	0.40	280	0.65	120	0.60
				485	0.60	150	0.45
						200	0.40
						300	0.35

tion of 500 IU per ml. For intramuscular use the same preparation containing 500 IU heparin and 1.25 mg carboxymethylcellulose per ml.

Dose

12 IU heparin per kg body weight.

Administration

Intravenously into a cubital vein. Intramuscularly at the locus electus in the upper gluteal region. Subcutaneously the anterolateral side of the thigh.

Blood sample

A sample of 10 ml blood was drawn from a superficial cubital vein by a clean puncture with a 0.8 mm gauge needle into a 10 ml polyethylene syringe after stasis for 10 seconds. When intravenous administration of heparin was used samples were drawn from the contralateral arm. Blood was stabilized with 5 IU heparin per ml immediately after drawing and at once centrifuged in the syringe for 3 minutes at 1400 × g at room temperature. Incubation was started within 6 minutes of the venepuncture.

Incubation

The incubation mixture was as described by Kern et al. (10): 1) 1.0 ml plasma, 2) 1.0 ml 0.05 M "tris" buffer (pH 8.8 37° C), 3) 100 mg bovine albumin (Fraction V powder, Armour) dissolved in 0.40 ml

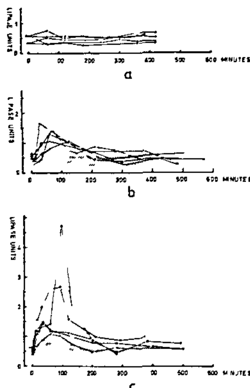


Fig 1 The lipoprotein lipase concentrations in plasma in five fasting normal test persons as a function of time 1 0 ml plasma is incubated with 1 5 ml substrate as described in text under Incubation The lipoprotein lipase activity is expressed in units as micromoles NEFA produces per ml plasma per 60 min under the assay conditions Fig 1 a The spontaneous lipolytic activity during a seven hour period. Fig 1 b The lipolytic activity in plasma after a dose of 12 IU heparin per kg body weight given subcutaneously Fig 1 c The lipolytic activity in plasma after the same dose given intramuscularly

NaOH (pH adjusted to 8 8 37 C) 4) 0 10 ml 15 per cent fat emulsion (Ediol R diluted with redistilled water) Before the addition of plasma the substrate mixture was preheated in the water bath at 37° C for at least 30 minutes

3 0 minutes after the addition of plasma a 'zero sample of 1 0 ml was taken for extraction of NEFA The enzyme activity was expressed in units as micromoles of NEFA produced per ml plasma per 60 minutes. For the determination of NEFA Dole s method (3), slightly modified was used Nile Blue A was found more convenient as an indicator than thymol blue NEFA was titrated with a Beckman Spinco microtitrator containing 0 100 N NaOH A blank was included in each set of extractions and the value found subtracted from the sample titration value To examine the reliability of the NEFA analysis known amounts of palmitic acid were added to the incubation mixture in concentrations from 250 to ± 15,000 micromoles per liter A recovery of 95 per cent ± 1 per cent was found

The analytical precision in duplicate incubations was ± 0 06 lipase units in the 95 per cent range

Results

The basal lipolytic activity was followed in all five test subjects for about seven hours The results are shown in table I and in fig 1 a The activity was rather constant during the day The average activity for all test subjects at the beginning of the test was 0 50 lipase units There was no rise during the prolonged fasting At the end of the test period the average activity was 0 48 lipase units The average spontaneous lipolytic activities before the heparin tests were 0 50, 0 58 0 46, 0 53, and 0 39 lipase units The highest spontaneous lipolysis registered was 0 65 units, the lowest 0 25 units The results of subcutaneous administration of heparin are shown in the table and in fig 1 b The lipolysis increased to about twice or thrice the pre heparin activity during the first and second hour The pre heparin values were usually reached after about three and a half hours In two cases a slight elevation was still found after about five hours

The rather varying lipolytic activity following intramuscular administration

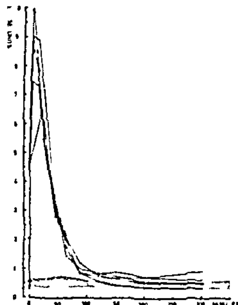


Fig 2 The lipoprotein lipase concentrations in plasma after an intravenous dose of 12 IU heparin per kg body weight Test conditions as described in fig 1

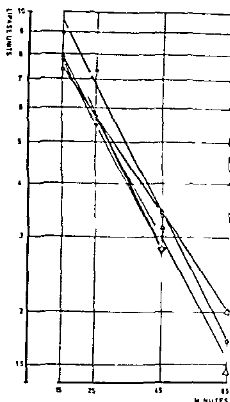


Fig 3 The linear decrease in lipoprotein lipase concentration in plasma between 15 and 60 min after intravenous heparin Test conditions as described in fig 1

of heparin is shown in fig 1 c and in the table The maximum activity was registered after 60 to 90 minutes and was from two to ten times the pre heparin level which was reached in about three and a half hours

Intravenous administration of heparin was followed by a very uniform lipolytic response as shown in the table and in fig 2 One minute after the injection an appreciable increase in the activity was seen and maximum activity was found between six and fifteen minutes after heparin At fifteen minutes the average activity was 8 lipase units (7 70 7 90 7 35, and 8 90, one measurement failed) After the maximum had been reached the activity decreased rapidly *The fall could be demonstrated* to be linear on a semilogaritmic scale, as shown in fig 3 From the activities found between 15 and 65 minutes the k value (slope constant) was calculated The average k value was 0 0138 (the individual measurements 0 0143 0 0141 0 0114 0 0149 and 0 0142)

Discussion

The basal lipolytic activity measured to provide a control for the evaluation of the post heparin lipoprotein lipase is not necessarily a parameter for the endogenous lipoprotein lipase activity in plasma However, experimental facts

may be considered circumstantial evidence that the lipolysis found is actually due to enzymic activity, the production of NEFA is completely stopped by adding to the incubation mixture protamine sulphate, a most potent lipoprotein lipase inhibitor (11). Furthermore, there is no detectable lipolysis in a system containing water instead of plasma. Lastly, the spontaneous lipolytic activity in plasma gradually decreases during storage at room temperature. In some samples the activity has completely disappeared after 24 hours. This fact may indicate lipolytic activity due to an unstable enzyme system, and is in accordance with the findings of Cseh and Szabo (2), who demonstrated a complete disappearance of lipoprotein lipase activity in serum after storage for *three hours*. However, recently we have found plasma samples, from apparently normal persons in which no decrease of the spontaneous lipolytic activity was found, even after storage at room temperature for 48 hours. This problem at present still remains to be explained.

The small, but significant rise in lipolytic activity after subcutaneous administration of heparin is not in agreement with the findings of Eiber et al. (5). These investigators found no lipolytic effect after a dose of about 5,000 IU subcutaneously. The discrepancy cannot be definitely explained from the paper of Eiber et al., but the possibility exists that it is due to the substrate used. Their incubation mixture consisted of 1 ml plasma and 0.2 ml 1.5 per cent coconut emulsion which renders a rather low substrate concentration. Furthermore they did not correct for pH and did not add albumin or *calciumions* for a NEFA acceptor, procedures considered necessary by most authors (1, 10, 11) for optimal measuring conditions.

Nikkila (12) used for subcutaneous administration a dose of 20,000 IU heparin and studied the lipolytic response by means of an in vitro turbidity test. Results obtained by this method are difficult to compare with results obtained by methods based on other principles. To this comes the much larger dose used by *Nikkila*. *However Nikkilä's* average curve shows an increase in the clearing capacity of plasma with a maximum after about two hours and a gradual decrease in enzymic activity during the next 22 hours. These *findings cannot be seen to disagree with the results reported in the present work.*

Engelberg (6) in a very extensive study of postheparin lipoprotein lipase generally used a dose of 20,000 IU heparin, in two cases 10,000 IU *heparin subcutaneously*. The lipase concentrations reported follow rather peculiar curves which cannot easily be interpreted. This may be due to an incomplete standardization of the *test method* as pointed out by the *author himself* and perhaps to some degree to a varying *resorption*. Still Engelberg found a rise in lipolytic activity in all cases though no consistent relation between dose and effect was seen.

In the present work it has been shown possible to get a uniform response to subcutaneous administration of heparin. The applicability of this route of administration should therefore not be denied as a therapeutic possibility.

Intramuscular administration of the combination of heparin and carboxymethylcellulose, "Heparin prolongatum", has been used at this department for almost 10 years with virtually no complications. The combination was therefore used for intramuscular dosage in the present study, and none of the five test persons developed heamatomas. The differences in lipolytic activity obtained seem to reflect differences in rate of absorption. Similar differences were found in the postheparin clotting time studies of Dollerup et al. (4). The two cases representing the highest lipolytic activity evidently follow curves for constant rate absorption and elimination (8). The others may represent intermediaries between absorption at constant rate and a constant per cent of the quantity, which at any time remains unabsorbed. However, no definite conclusion can be reached in the latter cases. The concentration of 2.5 μg carboxymethylcellulose per IU heparin was chosen after a test with 25 μg carboxymethylcellulose per IU heparin in one of the test persons. The latter preparation produced during a seven hour test period no detectable increase in lipoprotein lipase activity as compared to preheparin values.

Experiments have shown that carboxymethylcellulose per se has no effect on lipolysis, either when added to the incubation mixture in vitro, or when injected without heparin or in the opposite gluteal region at the same time as heparin.

The effect of carboxymethylcellulose consequently must be a local one at the site of injection of heparin. It is probably partly due to an osmotic effect, partly to the preparation being a potent anionic polyelectrolyte (9).

The results of lipoprotein lipase activity measurements after intravenous administration of heparin are in accordance with the findings published by Yoshitoshi et al. (14) and Boberg and Carlson (1). The slope of the curve of lipase concentrations between 15 and 65 minutes expressed as half life of the enzyme is in the present report on the average 21.9 minutes. Yoshitoshi et al. found an average half life of 24.5 minutes in eight normal men between 25 and 35 years of age. In the single curve presented by Boberg and Carlson the half life seems to be a little shorter.

I have found the most convenient expression to be the slope constant — the k value — of the best fitting semilogarithmic regression line based on the measurements of lipoprotein lipase concentrations in plasma found between 15 and 65 minutes after intravenous heparin, this expression being somewhat easier to calculate than the so called half life. The extrapolated zero value may be used in normal persons and is indeed an expression still easier to calculate and remember. However, findings in patients with disorders in lipid metabolism show combinations of varying post heparin lipase concentrations and slope constants which make the extrapolated zero value meaningless (7).

With an intravenous dose of 12 IU heparin per kg body weight a determination of the lipoprotein lipase concentration before heparin and for instance 15, 25, 35, 45, and 60 minutes after heparin, including a calculation of

the k value as discussed above, we hope to have found a tool useful in classifying disorders of lipid metabolism Further studies in this field are in progress

Summary

The lipolytic responses of plasma after a small dose of heparin (12 IU per kg body weight) by intravenous, subcutaneous, and intramuscular administration have been investigated

An in vitro test system has been used The spontaneous lipolytic activity is discussed A small but significant increase after subcutaneous administration is found

Intramuscular application has yielded varying results The very uniform activity after intravenous heparin is reported

A standardized lipoprotein lipase test is suggested

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Comparative Studies on the Diuretic and Biochemical Effects of Prednisone and Spironolactone in Hepatic Cirrhosis

By

J Lintrup, Th Friis and N I Nissen

The place of glucocorticoids, especially prednisone in the treatment of hepatic cirrhosis has been a matter of heated discussion Although the views have been conflicting it seems to have been generally accepted by now that a number of the biochemical parameters ordinarily used to assess the activity of a chronic parenchymal liver disease do improve on prednisone therapy This applies in particular to the serum transaminases (3 12 17, 23) Whether the disease process itself is favourably influenced is more doubtful, but this seems to be indicated by a few fairly recent studies (9 12, 13 30)

In addition prednisone often exerts a diuretic effect in the presence of oedema In hepatic insufficiency this effect is fairly constant, but is of limited degree and consists mainly in an increased excretion of water (7, 27, 32) Where the effect has been manifest in cardiac oedema, there has also been an increased excretion of sodium (15)

The aldosterone antagonist spironolactone has proved a good diuretic in hepatic oedema (8 18, 19 22) Spironolactone is of a steroid structure and in recent experiments on rats it has shown unmistakable anti inflammatory properties (2) This raises the question whether spironolactone like prednisone also has a favourable effect upon the biochemical tests in diseases of the liver If so the next question is whether this favourable effect is merely secondary to an improvement in the general condition after excretion of the oedema Conversely, it may be asked in the case of prednisone whether the mild diuretic effect in these cases is merely secondary to an improvement in the condition of the hepatic parenchyma In an effort to throw additional light upon these problems, we followed the excretion of sodium and the biochemical parameters in some patients with chronic hepatic diseases during successive treatment with spironolactone and prednisone, in

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TABLE I Average output of sodium (mEq/24 hrs) in 6 patients with hepatic cirrhosis and severe oedema

Case no	Before treatment mEq sodium/24 hrs	On spironolactone mEq sodium/24 hrs	Off treatment mEq sodium/24 hrs	On prednisone mEq sodium/24 hrs	On spironolactone + prednisone mEq sodium/24 hrs
1	8	[1]96	5	35	[2]215
				7	[1]129
				11	
2	50	38	16	50	[2]108
3	12	[1]89	25	22	[1]89
4	25	[1]79	4	3	[2]80
		[1]79	32		
5	32	[1]219	91	[1]164	
6	9	[1]93	17	1	[2]52

[1] Natriuretic effect sodium output > 200% of the mean value during periods off treatment and > 50 mEq/24 hours

[2] Potentiated natriuretic effect sodium output > 150% of the value during the spironolactone period

some of the cases also during combined treatment with both agents

Material and methods

The investigations refer to 18 patients aged 36—83 years 7 males and 11 females All were in patients during the period of the study Six had a history of alcohol abuse Six had severe oedema and pronounced *ascites* 6 had *moderate oedema and no* definite ascites while the last 6 had no manifest oedema All exhibited the clinical picture of active hepatic cirrhosis However two had severe acute symptoms at the outset. The chronic nature of the disease in one of these patients was established by liver biopsy and in the other by repeated biochemical recurrences through 18 months

Spironolactone was administered in the form of Aldactone® (Searle) 225 mg daily divided into three doses Aldactone was used in its new micronized form in which 25 mg corresponds to 100 mg of the original preparation The dose of prednisone was 30 mg daily also divided into three doses After a preliminary period of one week without treatment the patients entered treatment periods of two weeks interrupted by one week off treatment It was not possible in all cases to observe this programme accurately Two patients were treated with prednisone before spironolactone and 13 with spironolactone first Nine were given final period on combined treatment with both drugs

The diet was our light diet without a supply of salt and containing about 80 mEq sodium daily No substances having a diuretic effect and no supplement of electrolytes of any kind were given

The 24 hour outputs of urine sodium and potassium were determined daily (Eppendorff flame photometer) Every week the patients were weighed and the blood volume as well as sodium pool determined The blood volume was determined by ^{131}I-labelled albumin by means of a volemetrone apparatus (Atomium Perkin Elmer) (21, 24 31) About 3 μC was administered for

TABLE II Average output of sodium (mEq/24 hrs) in 6 patients with hepatic cirrhosis and moderate oedema

Case no	Before treatment mEq sodium/24 hrs	On spironolactone mEq sodium/24 hrs	Off treatment mEq sodium/24 hrs	On prednisone mEq sodium/24 hrs	On spironolactone prednisone mEq sodium/24 hrs
7	[illegible]	[illegible]	74	53	87
8	78	94	33	84	
9		109	41	36	[1]16
10	111	105	48		
11	76	124	110	150	13
12	22	[1]121			133

[1] Natriuretic effect sodium output [illegible] 200 % of the mean value during periods off treatment and > 50 mEq/24 hours

[2] Potentiated natriuretic effect sodium output [illegible] 150% of the value during the spironolactone period

TABLE III Average output of sodium (mEq/24 hrs) in 4 patients with hepatic cirrhosis with oedema

Case no	Before treatment mEq sodium/24 hrs	On spironolactone mEq sodium/24 hrs	Off treatment mEq sodium/24 hrs	On prednisone mEq sodium/24 hrs	On spironolactone prednisone mEq sodium/24 hrs
13	99	86	53	117	
			124		
14	91	108	94		
15	45	6	56	84	
		[1]85	21		
16	90	79	53		

[1] Natriuretic effect sodium output > 200 % of the mean value during periods off treatment and > 50 mEq/24 hours.

every test. The *coefficient of variation* for the measurements was 2–4 %. No regard was paid to the difference between peripheral haematocrit and total haematocrit. Exchangeable sodium was determined by the short lived isotope ^{24}Na ($t\ 1/2 = 15$ h) with a 24 hour equilibration period (16). The sodium pool determined thereby makes up only 75 % of the total sodium content of the body, the sodium in the bones not being included. About 50 μC was administered for each determination. The coefficient of variation for the measurements was 2–5 %.

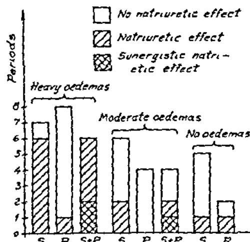


Fig 1 Natriuretic effect in various degrees of oedema S = Spironolactone P = Prednisone S + P = Spironolactone + prednisone

For assessing the activity of the liver disease the following biochemical studies were carried out twice weekly serum glutamic acid oxalacetic acid transaminase GOT thymol turbidity Takata Ara alkaline phosphatases icterus index Meulengracht prothrombin Owren's PP method serum albumin electrophoresis and erythrocyte sedimentation rate 1 hour

In two of the patients it was impossible to carry through the measurements of the urinary excretion because of difficulties in collecting the urine

Results

The average sodium excretion in the different periods is shown in tables I—III On the reckoning that the criterion of a natriuretic effect is a minimum increase in the 24 hour output to 200 % of the average output during the periods off treatment and exceeding 50 mEq/24 hours the diuretic effect in the various degrees of oedema is apparent from fig 1 This figure also illustrates the number of periods with a synergistic diuretic effect of the combination spironolactone and prednisone The criterion of synergism was an increase to more than 150 % of the excretion observed during the periods on spironolactone alone

It will be seen that a natriuretic effect of spironolactone was found in 6 out of 7 cases with severe oedema, in 2 out of 6 with moderate oedema, and in 1 out of 5 with no oedema With prednisone an effect was obtained in only 2 cases, one in the group with severe oedema and the other in a patient without oedema However, prednisone acted synergistically with spironolactone in 3 cases in which it had been ineffective alone

During spironolactone therapy there was in most cases a fall of serum sodium but only to values around the lower limit of normal On the other hand one third of the patients developed quite considerable hyperpotassaemia, which thus in this series, constituted a more serious complication than the hyponatraemia Apart from this we have not observed side effects except for a fairly common tendency to diarrhoea towards the end of the treatment period

Fig 2 illustrates the concurrent values for the sodium pool and blood volume both stated per kg body weight This mode of expression is most widely used, but far from ideal There is a considerable spread of the normal values expressed in this way and there is disagreement concerning the normal range (4, 5 6, 10, 16, 21, 24, 28) The limits marked on fig 2 are 39—48 mEq/kg for the sodium pool (16) and 60—95 ml kg for the blood volume

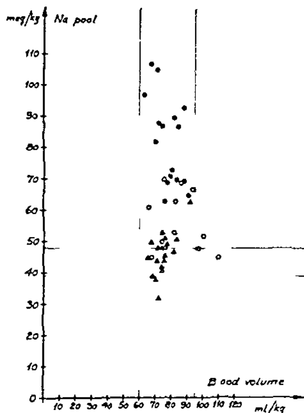


Fig. 2. Concurrent values of exchangeable sodium and blood volume. ● In the presence of considerable ascites. ○ In the presence of moderate oedema. △ No manifest oedema.

The group of patients with ascites showed a highly increased sodium pool while the blood volume was normal. In the presence of more moderate oedema the values varied greatly without there being a correlation between the two parameters, while in the group of patients without oedema there was a positive correlation ($r = 0.64$, $P < 0.01$).

In table IV the overall pattern of biochemical liver tests during the observation periods is classified as improved, unchanged or worse. To classify a case as improved or worse during an observation period we demanded concordant clear alterations in at least two tests without a definite conflict with the other tests. The classification was done after the investigations were completed, collected for all 80 periods and without a knowledge of the patient's identity or the nature of the treatment. Thus the assessment is objective in the sense that it is uniform and unaffected by a knowledge of the nature of the treatment, but inherently it must depend upon an estimate. During the periods off treatment there was a great preponderance of an unchanged biochemical pattern and during spironolactone medication a tendency towards biochemical improvement may be traced, a tendency which becomes very clear during the periods

TABLE IV Assessment of the total liver function tests during spironolactone and prednisone therapy

	Total periods	Average duration (days)	Improved	Unchanged	Worse
Untreated	34	8	4	25	5
Spironolactone	20	18	9	9	2
Prednisone	16	15	11	4	1
Spironolactone + prednisone	10	16	2	8	0
Total	80		26	46	8

TABLE V Comparison of the natriuretic and biochemical effects of spironolactone and prednisone

	Total periods	+ natriuresis + biochemical effect	+ natriuresis no biochemical effect	No natriuresis + biochemical effect	No natriuresis no biochemical effect
Spironolactone	18	2 (11%)	7 (39%)	6 (33%)	3 (17%)
Prednisone	14	2 (14%)	0 (0%)	7 (50%)	5 (36%)
Spironolactone + prednisone	10	2 (20%)	6 (60%)	0 (0%)	2 (20%)
Total	42	6 (14%)	13 (31%)	13 (31%)	10 (24%)

on prednisone During combined medication there was no instance of biochemical aggravation, but there was no clear trend

For 42 out of the 46 treatment periods a comparison could be made of biochemical and natriuretic effects It will be seen from table V that treatment periods in which only one effect was manifest are in majority (62 %), equally distributed on natriuretic and biochemical effect Periods without any effect (24 %) or with an effect in both respects (14 %) were in a minority (38 %) It must be mentioned that in 6 instances a biochemical effect was recorded on spironolactone without a simultaneous natriuretic effect and that in no case was a natriuretic effect recorded during prednisone therapy without a simultaneous biochemical effect

Out of the 6 patients with a history of alcohol abuse, 2 had severe ascites, 2 had moderate oedema and 2 no oedema A separate analysis of the results in these 6 cases failed to disclose any items in which the results differed essentially from those in the total series

Discussion

The results confirm the effect of spironolactone in counter-acting oedema and the effect of prednisone upon the bio-

chemical parameters in chronic liver diseases The combination of prednisone and spironolactone showed an even more reliable natriuretic effect than *spironolactone alone whereas treatment* with prednisone alone seldom had this effect The explanation can hardly be that in most cases prednisone was administered during a later treatment period than spironolactone, as the good results of combined therapy were obtained in all cases during the last treatment period Other workers have also found a synergistic diuretic effect during combined medication with prednisone and spironolactone (11, 26) The mechanism of the action of prednisone in this connection is not understood However it has been demonstrated that hyperaldosteronism in the presence of hepatic cirrhosis with ascites may be due to an increased production (1, 20 29) as well as to delayed catabolism of aldosterone (1, 14, 20, 33) Perhaps prednisone may *via* a pituitary suppression inhibit that part of the hyperproduction of aldosterone which is possibly dependent upon ACTH (7, 25) and thereby support the aldosterone antagonism of spironolactone

In the presence of ascites there was invariably a highly increased sodium pool, but a normal blood volume *(fig 2) This is in keeping with the* findings of others (except for a moderate increase in the presence of advanced oesophageal varices) (10, 28) and unlike the findings in ascites due to cardiac decompensation in which case the blood volume was highly increased too In the presence of more moderate oedema the patients showed highly varying relations between the sodium pool and the blood volume In most instances the sodium pool was moderately increased without a corresponding *increase in the blood volume In a few* cases there was an increased blood volume without the same relative increase in the sodium pool Thomas and Bartter (28) have suggested that in these patients ascites develops later at the expense of the plasma volume Where no manifest oedema was present the sodium pool as well as the blood volume was generally within the normal range, and there was a weakly positive correlation between the values

In assessing the alterations in the total picture of the liver tests (table IV) regard must be paid to the fact that the treatment periods were longer than the control periods This may partially explain why a relatively large number of the latter fall within the group with an unchanged pattern but does not influence the relation between periods with improvement and aggravation As might be expected there is a great preponderance of improvement during prednisone therapy But the spironolactone periods too may show a preponderance of improvement compared with the control periods The results of combined therapy may appear less *favourable but it must be borne in* mind that the combined therapy was invariably preceded by a fairly long lasting treatment with the drugs separately However there was not in any case an aggravation of the biochemical pattern during the combined therapy

According to table V there does not appear to be any correlation between

that which we have accepted in this material as a natriuretic effect and that which we have accepted as a biochemical effect An equal number of periods with a favourable effect was recorded in each of these respects If an effect in one respect should occur as a consequence of the effect in the other, it might be expected that the material would show a preponderance of cases with or without an effect in both respects This was not so On the contrary there was a slight preponderance in groups where an effect was found only in one respect In particular spironolactone was recorded as having a biochemical effect in 6 of the 9 periods without a natriuretic effect

Thus, the improvement of the biochemical parameters on spironolactone indicated by the values found in the present series cannot be explained as a consequence of a diuretic effect Such an explanation would also not have fitted the experience that an aggravation increasing to hepatic coma, may be elicited by an energetic diuretic therapy On the other hand, a biochemical improvement may be explained on the basis of Bajusz and Jasmin s hypothesis according to which spironolactone exerts an anti inflammatory effect by suppressing the pro inflammatory activity of endogenous mineralocorticoids 2

Summary

In 18 patients with active cirrhosis of the liver an attempt was made to compare the diuretic and the biochemical" effects during treatment with the aldosterone antagonist spironolactone and with prednisone for two week periods Nine of the patients also had combined treatment with both drugs The results confirm the good diuretic effect of spironolactone and the favourable effect of prednisone upon the biochemical parameters In 3 of the patients the natriuretic effect of spironolactone was increased by simultaneous administration of prednisone The significance of this synergism is discussed

Rather surprisingly, the results also indicated a favourable effect of spironolactone upon the biochemical parameters, and an analysis of the material revealed that such an effect could not be due to its diuretic activity

Acknowledgement

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PHILIP HENCH

In Memoriam

By Nanna Svartz

Philip Showalter Hench Nobel Prizewinner died a short time ago at the age of 69 while on holiday in Jamaica

Together with the chemists Edward Kendall — employed as was Hench at the Mayo Clinic Rochester Minnesota — and Tadeus Reichstein of the Basel University Hench was awarded the Nobel Prize for Physiology and Medicine for his discovery concerning the cortical hormones their structure and biological effects

As early as 1925 Hench had published a paper in which he pointed out that chronic arthritis now called rheumatoid arthritis was probably attended by pronounced metabolic changes He was struck particularly by the common occurrence of low blood pressure in this disease and assumed partly on this account that the adrenal glands might be involved but despite numerous attempts he could find no definite changes in these organs He therefore directed his studies elsewhere to the possible influence of the liver on the course of chronic arthritis He had observed several cases of jaundice in chronic arthritis patients and their arthritic symptoms had manifestly improved as long as the jaundice lasted Hench now assumed that there was an antirheumatic element in the liver and examined a large number of substances occurring in the liver and bile but with negative result.

In the early thirties Hench began to investigate the circumstance observed also by others that patients with chronic arthritis who became pregnant often experienced a considerable improvement of their arthritic symptoms There must thought Hench, be a lack of some substance in the organism, probably a hormone He believed at first that this substance was merely antirheumatic but it soon became clear that pregnancy could also have a favourable effect on hay fever asthma migraine and other conditions Substance X he assumed therefore, was specific to a group of diseases and not to a particular disease This proved assuredly to be a true prediction

Hench and Kendall often discussed different medical products and in 1941 they decided to try compound E later called cortisone in arthritis when it became available in sufficient quantities Kendall had isolated an insignificant quantity of this cortical hormone in 1934 in collaboration with Mason and Myers Reichstein was engaged on the same problem

The first quantities of the hormone had been reserved for patients with adrenal insufficiency In September 1948 Hench wrote to Merck & Co , where Sarett in collaboration with Kendall and others had started to synthesize cortical hormones He asked for a quantity of hormone E large enough at least for trial on one patient At the end of that month he and his coworkers received a quantity which was given to a patient who had had rheumatoid arthritis for four years accompanied by serious loss of motility After five days of treatment the

(Cont page 28)

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From the Medical Department VIII (Head: Anton Jervell, M. D.) and the Department of Pathology (Head: Kristen Arnesen, M. D.), Ullevål Hospital, Oslo, Norway

Electrocardiogram in Patients with Healed Myocardial Infarction Disclosed at Autopsy

By

Øyvind Skjæggestad and Kåre Molne

In an earlier investigation (1) one of the authors studied the ECG findings in patients with previous clinically diagnosed myocardial infarction (m. i.). Originally 82 per cent of these patients had unequivocal ECG signs of an acute m. i., which is in accordance with other figures given, varying from 70 per cent (15) to about 90 per cent (5). One half to two years later the unequivocal ECG signs of m. i. disappeared in 16 per cent (1), thus 31 per cent of the 128 patients studied failed to show definite ECG signs of previous m. i. These results are in accordance with those found by other investigators (2, 6), some however have found unequivocal signs of previous m. i. in all but 2 per cent of the patients (7, 13).

When ECG findings are compared with autopsy findings of old m. i. a much lower incidence of unequivocal ECG infarction signs are seen. Woods et al. (16) and Diederich et al. (4) both found ECG evidence of old infarction in only 38 per cent, Levine et al. (9) in 20 per cent, while Melichar et al. (12) found it in only a few per cent. In the last mentioned study very few ECG leads however were taken. The explanation of this great discrepancy between pure clinical studies and those verified at autopsy might be that the latter studies also comprise clinically unrecognised m. i. Johnson et al. (8) found that in only 50 per cent of healed infarcts found at autopsy had the condition been clinically diagnosed or suspected. Other investigations have also revealed a high incidence of unrecognized healed infarcts (9, 14). The clinical diagnosis of m. i. may easily be overlooked when precordial pains are absent or very slight. In such cases the infarct usually is small, patchy or subendocardial and unequivocal ECG signs will probably be rarer.

Autopsy studies of acute m. i. correlated to the ECG findings do not give a correct picture of the above mentioned problems since patients with small and subendocardial m. i. usually survive the

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PHILIP HENCH

In Memoriam

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Together with the chemists Edward Kendall — employed as was Hench at the Mayo Clinic Rochester Minnesota and Tadeus Reichstein of the Basel University Hench was awarded the Nobel Prize for Physiology and Medicine for his discovery concerning the cortical hormones their structure and biological effects

As early as 1925 Hench had published a paper in which he pointed out that chronic arthritis now called rheumatoid arthritis was probably attended by pronounced metabolic changes He was struck particularly by the common occurrence of low blood pressure in this disease and assumed partly on this account that the adrenal glands might be involved but despite numerous attempts he could find no definite changes in these organs He therefore directed his studies elsewhere to the possible influence of the liver on the course of chronic arthritis He had observed several cases of jaundice in chronic arthritis patients and their arthritic symptoms had manifestly improved as long as the jaundice lasted Hench now assumed that there was an antirheumatic element in the liver and examined a large number of substances occurring in the liver and bile but with negative result

In the early thirties Hench began to investigate the circumstance observed also by others that patients with chronic arthritis who became pregnant often experienced a considerable improvement of their arthritic symptoms There must, thought Hench, be a lack of some substance in the organism, probably a hormone He believed at first that this substance was merely antirheumatic but it soon became clear that pregnancy could also have a favourable effect on hay fever asthma migraine and other conditions Substance X he assumed therefore was specific to a group of diseases and not to a particular disease This proved assuredly to be a true prediction

Hench and Kendall often discussed different medical products and in 1941 they decided to try compound E later called cortisone in arthritis when it became available in sufficient quantities Kendall had isolated an insignificant quantity of this cortical hormone in 1934 in collaboration with Mason and Myers Reichstein was engaged on the same problem

The first quantities of the hormone had been reserved for patients with adrenal insufficiency In September 1948 Hench wrote to Merck & Co where Sarett in collaboration with Kendall and others had started to synthesize cortical hormones He asked for a quantity of hormone E large enough at least for trial on one patient At the end of that month he and his coworkers received a quantity which was given to a patient who had had rheumatoid arthritis for four years accompanied by serious loss of motility After five days of treatment the

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From the Medical Department VIII (Head: Anton Jervell, M. D.) and the Department of Pathology (Head: Knuten Arnesen, M. D.), Ullevål Hospital, Oslo, Norway

Electrocardiogram in Patients with Healed Myocardial Infarction Disclosed at Autopsy

By

Øyvind Skjæggestad and Kåre Molne

In an earlier investigation (1) one of the authors studied the ECG findings in patients with previous clinically diagnosed myocardial infarction (m. i.). Originally 82 per cent of these patients had unequivocal ECG signs of an acute m. i., which is in accordance with other figures given, varying from 70 per cent (15) to about 90 per cent (5). One half to two years later the unequivocal ECG signs of m. i. disappeared in 16 per cent (1); thus 31 per cent of the 123 patients studied failed to show definite ECG signs of previous m. i. These results are in accordance with those found by other investigators (2, 6); some, however, have found unequivocal signs of previous m. i. in all but 2 per cent of the patients (7, 13).

When ECG findings are compared with autopsy findings of old m. i., a much lower incidence of unequivocal ECG infarction signs are seen. Woods et al. (16) and Diederich et al. (4) both found ECG evidence of old infarction in only 38 per cent, Levine et al. (9) in 20 per cent, while Melcher et al. (12) found it in only a few per cent. In the last mentioned study very few ECG leads, however, were taken. The explanation of this great discrepancy between pure clinical studies and those verified at autopsy might be that the latter studies also comprise clinically unrecognized m. i. Johnson et al. (8) found that in only 50 per cent of healed infarcts found at autopsy had the condition been clinically diagnosed or suspected. Other investigations have also revealed a high incidence of unrecognized healed infarcts (9, 14). The clinical diagnosis of m. i. may easily be overlooked when precordial pains are absent or very slight. In such cases the infarct usually is small, patchy or subendocardial, and unequivocal ECG signs will probably be rarer.

Autopsy studies of acute m. i. correlated to the ECG findings do not give a correct picture of the above-mentioned problems since patients with small and subendocardial m. i. usually survive the

Submitted for publication June 2[illegible], 196[illegible]

PHILIP HENCH

In Memoriam

By Nanna Svartz

Philip Showalter Hench Nobel Prizewinner died a short time ago at the age of 69 while on holiday in Jamaica

Together with the chemists Edward Kendall — employed as was Hench at the Mayo Clinic Rochester Minnesota — and Tadeus Reichstein of the Basel Universi ty Hench was awarded the Nobel Prize for Physiology and Medicine for his discovery concerning the cortical hormones their tructure and biological effects

As early as 1925 Hench had published a paper in which he pointed out that chronic arthritis now called rheumatoid arthritis was probably attended by pronounced metabolic changes He was struck particu larly by the common occurrence of low blood pressure in this disease and assumed partly on this account that the adrenal glands might be involved but despite numerous attempts he could find no definite changes in these organs He therefore directed his studies elsewhere to the possible influence of the liver on the course of chronic arthritis He had observed several cases of jaundice in chronic arthritis patients and their arthritic symptoms had manifestly improved as long as the jaundice lasted Hench now assumed that there was an antirheumatic element in the liver and examined a large number of substances occurring in the liver and bile but with negative result

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In an earlier investigation (1) one of the authors studied the ECG findings in patients with previous clinically diagnosed myocardial infarction (m i) Originally 82 per cent of these patients had unequivocal ECG signs of an acute m i which is in accordance with other figures given varying from 70 per cent (15) to about 90 per cent (5) One half to two years later the unequivocal ECG signs of m i disappeared in 16 per cent (1), thus 31 per cent of the 128 patients studied failed to show definite ECG signs of previous m i These results are in accordance with those found by other investigators (2, 6), some, however have found unequivocal signs of previous m i in all but 2 per cent of the patients (7 13)

When ECG findings are compared with autopsy findings of old m i a much lower incidence of unequivocal ECG infarction signs are seen Woods et al (16) and Diederich et al (4) both found ECG evidence of old infarction in only 38 per cent, Levine et al (9) in 20 per cent while Melichar et al (12) found it in only a few per cent In the last mentioned study very few ECG leads however were taken The explanation of this great discrepancy between pure clinical studies and those verified at autopsy might be that the latter studies also comprise clinically unrecognised m i Johnson et al (8) found that in only 50 per cent of healed infarcts found at autopsy had the condition been clinically diagnosed or suspected Other investigations have also revealed a high incidence of unrecognized healed infarcts (9 14) The clinical diagnosis of m i may easily be overlooked when precordial pains are absent or very slight In such cases the infarct usually is small patchy or subendocardial and unequivocal ECG signs will probably be rarer

Autopsy studies of acute m i correlated to the ECG findings do not give a correct picture of the above mentioned problems since patients with small and subendocardial m i usually survive the

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TABLE I ECG findings in 134 patients with healed myocardial infarcts disclosed at autopsy A. Unequivocal ECG signs B Equivocal ECG signs C Pathological ECG but without signs of previous myocardial infarction D Normal ECG

	Bundle branch block (BBB)	Left ventricular hypertrophy (LVH)	No signs of BBB or LVH	Total
A	5	21	21	47
B	21	13	11	45
C	0	32	7	39
D	0	0	3	3

acute episode The purpose of the present study was to investigate the ECG findings in patients with old m i disclosed at autopsy, using the same criteria for the ECG interpretation as in the clinical study mentioned above (1)

Material and method

The 134 patients included in this study died in the medical departments VII VIII and IX of Ullevål Hospital Oslo during the years 1960 to 1963 They all had a) a healed m i at autopsy b) no signs of recent myocardial necrosis and c) a 12 lead ECG recorded at least once during the last month prior to death

By the *routine autopsy technique* the pericardium was opened the aorta and the pulmonary artery were cut 2—3 cm above the semilunar valves and the great veins were cut near the junction with the heart The heart was then opened with scissors along the septum following the direction of the blood flow The endocardium was inspected for pathological changes the myocardium of the left ventricle was sliced and carefully inspected for necrotic and fibrous lesions The coronary tree was opened with fine scissors and search was made for stenoses and occlusions Finally the heart was weighed

Fibrous myocardial scars with a diameter exceeding 1 cm were considered to represent an old infarct The criteria for myocardial hypertrophy were heart weights above 400 g (females) or above 450 g (males)

The *ECG findings* were classified as follows A ECG with unequivocal signs of infarction pathological Q waves, in doubtful cases supplemented by ST T changes were considered as evidence of infarction The criteria were adopted from Lipman and Massie (10) B ECG with equivocal signs of infarction the above mentioned criteria were not met in this group but ECG was not inconsistent with the possibility of previous infarction C ECG pathological, but with no signs of infarction D ECG normal

Bundle branch block (BBB) and left ventricular hypertrophy (LVH) were also registered according to the criteria adopted from Lipman and Massie (10)

Results and discussion

Data obtained from routine autopsy must be considered with some care However, some information can be relied upon at least one healed m i was found in each heart The description of the location of the scars is assumed to be correct, the main purpose in slicing the myocardium is to search for fibrous and necrotic lesions The location of the infarcts is seen from table II and is commented upon below Unfortunately the autopsy description made no distinction between transmural and subendocardial infarcts The heart weight must also be considered correct, the same technique of removing the heart and weighing the organ having

TABLE II The location of the myocardial infarcts found at autopsy correlated to the ECG findings during the last month prior to death A Unequivocal ECG signs B Equivocal ECG signs. C. Pathological ECG but with no signs of previous myocardial infarction D Normal ECG

	Non septal				Septal				
	Anterolateral l. ventr. wall	Posterior l. ventr. wall	Uncertain location in l. ventr. wall	Total	Anterolateral l. ventr. wall and septum	Posterior l. ventr. wall and septum	Septum only	Uncertain location in l. ventr. wall and septum	Total
A	12	8	0	20	7	6	8	6	27
B	15	3	4	22	10	0	6	7	23
C	18	9	1	28	2	4	4	1	11
D	0	2	0	2	1	0	0	0	1
Total	45	22	5	72	20	10	18	14	62

been used for years in this laboratory *In 80 per cent of the patients an enlargement* of the heart was found, the median weight being 505 g (range 280—1,290)

From table I is seen that 47 (35 per cent) of the patients had ECG evidence of an old infarction Twenty-one of these were anterior (Q in I aVL or/and V_{1-4}) and 26 posterior (Q in II III or/and aVF) *These locations were* confirmed at autopsy in all but one anterior, while 7 patients with ECG signs of posterior infarction had a scar in the anterior or lateral wall A fibrous lesions in the posterior wall (in addition to anterior and lateral walls) might have been overlooked

Equivocal ECG signs of m. i. were seen in 45 patients Thirty four of them had BBB or LHV in the ECG, and in 29 cases this was the main cause of the ECG being non-diagnostic for m. i. In the remaining 16 patients small R and Q waves were *the* cause of uncertainty In the whole material there were 92 patients with and 42 patients without BBB or LVH in the ECG The incidences of unequivocal ECG signs of m. i. in the two groups were 26 (28 per cent) and 21 (50 per cent) respectively All patients with ECG signs of LVH (66 cases) had hypertrophied hearts at *autopsy according to the criteria used* except 4 patients with heart weights from 370 to 400 g On the other hand half of the patients with no signs of LVH had enlarged hearts

No signs of m. i. in the ECG were seen in 42 patients (31 per cent) Also in this group of patients LVH signs in the ECG may have obscured the signs of previous infarction A normal ECG was only seen in 3 patients (2 per cent) which is in accordance with other investigations (9 11, 17)

In table II the locations of the infarcts found at autopsy are correlated to the ECG findings It is seen that unequivocal ECG signs of m i are more often seen when the infarcts also involve the septum (44 per cent) than when they do not 28 per cent) Unequivocal signs are also more often seen when the infarcts are localized to the posterior ventricular wall rather than to the anterior or lateral wall

When the results in the present study are compared with those in the clinical study mentioned above (1) it is evident that unequivocal ECG signs of an old m i were seen in 69 per cent of the patients in the clinical study but only in 35 per cent in the autopsy study The two investigations have been performed at different hospitals but the ECG criteria were the same and the interpretations were made by the same author Though observer variations can be great 3) the considerable divergence between the two investigations cannot be explained in this way Regressive ECG changes apparently take place within the first 1/2 to 1 year after the infarct 1 4 later, however BBB and LVH changes may occur in the ECG and make the interpretation more difficult Again the great difference cannot be thus explained Even if all patients in the autopsy material with BBB and LVH were excluded from the comparison the frequency of unequivocal ECG signs of m i in the clinical material would be much greater The most important explanation of the discrepancy therefore probably is a high frequency of clinically unrecognized m i where unequivocal ECG signs of m i often are lacking Thus m i may be still more frequent, and the ECG interpretation less accurate, than is ordinarily assumed

Summary

A study of the ECG findings in 134 patients in whom an old myocardial infarct was disclosed at autopsy has been made None of the patients died from coronary heart disease, in all of them however, a 12 lead ECG had been recorded at least once during the last month prior to death

In 47 patients (35 per cent) the ECG showed unequivocal signs of previous myocardial infarction (m i), in 45 patients (34 per cent) there were equivocal signs and in 42 patients (31 per cent) there was no evidence of old infarction in the ECG, but in only 3 patients (2 per cent) were the ECGs considered normal

The study showed further that infarcts comprising the septum and posterior ventricular wall could more frequently be detected than those localized to the antero lateral ventricular wall

ECG signs of left ventricular hypertrophy (LVH were found in 66 patients and of bundle branch block (BBB) in 26 patients In only 26 (28 per cent) of these 92 patients did the ECG disclose unequivocal signs of m i Among the remaining 42 patients without ECG signs of LVH or BBB, 21 (50 per cent) had unequivocal signs Thus together with regressive changes BBB and LVH changes make the ECG diagnoses of previous m i difficult

The discrepancy between the present study and an earlier clinical study (1) which had a much higher frequency of unequivocal ECG signs of previous m. i., makes it rather likely that there is a high incidence of clinically unrecognized acute myocardial infarcts.

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patient said I never felt better in my life ' The news of what had happened at the Mayo Clinic spread like wildfire across the world despite all attempts by Hench and his colleagues, Slocumb and Polley, to hush it up until they had tried out the compound thoroughly Within a few days they realized that the arthritic symptoms recurred if the medication was interrupted This later gave rise to endless discussions concerning the possible advantages of long time treatment or of larger doses of cortisone Attempts in the use of large doses led to the soon widely known side effects and even resulted in prohibition of the use of the drug in some clinics This pained Hench deeply As so often when expectations run high the reaction was altogether too violent Hench himself had been very reserved in his judgment and often emphasized that more experience was needed and that one had to proceed with caution After he had thoroughly tried out the compound he issued detailed directives for the treatment His principles are at present being followed to a large extent everywhere in the world

If one asks what were the results of the experiments made by Hench and his colleagues some 17 years ago it must first be pointed out that cortisone and allied compounds had started to be synthesized by chemists and drug manufacturers solely for use in cases of adrenal insufficiency Here Selye s studies of the significance of the adrenal glands enter the picture The persevering and fundamental studies of Hench Kendall Slocumb and Polley immediately showed that cortisone could have a marked effect in other than adrenal diseases proper Cortisone soon became an indispensable aid in rheumatoid arthritis and other diseases of the connective tissue allergic diseases blood diseases, tumours and so on Hench may be denoted the prime mover in this enormous and rapid development Since we have learnt the technique of low dosage and local treatment, serious side effects have become increasingly rare Cortisone and allied compounds have proved to have an unusually fruitful influence also on research The main point, however is that millions and millions of people with severe diseases of different organs are finding and will continue to find relief through these and similar chemical compounds This development started in the Mayo Clinic, September 1948

Philip Hench devoted himself with a rare enthusiasm and indomitable energy both to his daily medical practice and to his important research He was scrupulously thorough and completely honest and sound in all his work He was a highly intelligent person, a profound thinker and a constant seeker after knowledge, always pondering over new problems which he often wished to discuss all the night long He was a good man faithful in his friendship Relaxation from his work he found in his family circle and in music or in his large library of medical and other literature

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From the Department of Medicine (Head U Sagild M D) Dronning Ingrids Hospital Godthaab Greenland

Epidemiological Studies in Greenland 1962—1964

I Diabetes mellitus in Eskimos

By

Uffe Sagild[1] Jorgen Littauer C Sand Jespersen and S Andersen

The rarity of diabetes mellitus in Eskimos was first noted by Bertelsen (1), who suggested that this was more apparent than real and probably the result of inadequate diagnostic facilities

However in a survey of 1,227 Alaskan Eskimos of both sexes Scott and Griffith (14) found only three individuals with elevated fasting blood sugar levels, two of them being half Eskimos An inquiry of the eight hospitals of Alaska serving an Eskimo population of 16 000 disclosed three further confirmed cases of diabetes Two of these were related and all three were from the vicinity of Nome, where many Eskimos of mixed origin are living

In a recent population survey of 1 500 Canadian Eskimos Davies and Hanson (4) tested urines of 637 individuals for glucose Although twenty four specimens gave a positive test for glucose physical, radiographic and blood studies on the individuals from whom these urines were obtained revealed no abnormality Two Eskimos living in the area surveyed, a woman aged 54 and a man aged 41 years were known to have diabetes mellitus

In Greenland which is a county of Denmark 92 per cent of the population (approx 35 000) is of mixed Danish Eskimo descent or is pure blooded Eskimo No data concerning the prevalence of diabetes mellitus in this population have been published so far

The present report summarizes the results of a population survey comprising 4 249 individuals This survey begun in 1962 and concluded in 1964 was carried out in three different areas of Greenland In addition the total hospital experience of diabetes mellitus in Greenland was reviewed

Methods

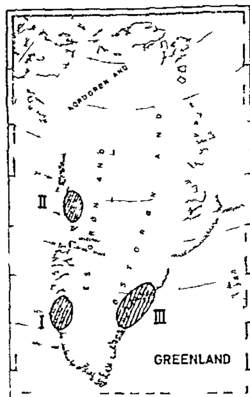
Population survey

The three areas chosen for the survey were the following (fig 1)

I *The Godthaab-area* excluding the capital Godthaab The population of this area is

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F g 1 The areas n Greenland chosen for the surve

about 1 500 distribu ed o er six villages at the southern part of the west coast of Green land Lat 63—64 N The main occupat on is fish ng and the predom nant d et is f sh products supplemented by imported farm products, potatoes etc Thus the compos t on of the diet does not d ffer greatly from the a era e d ets consumed in Denmark proper The people are mostl of mixed Eskimo-Danish origin with few f any pure blooded Esk mos

II *The Umanak a ea* excluding the main town Umanak The population of th s area is approx. 1 500 inhab tants l ing in seven small villages alon the coast of the Umanak fjord on the northern part of the west coast (Lat. 0—72 N Here whale and seal hunting is a major occupation together with fishing Thus a large part of the caloric intake — and periodically the only source of dietary intake — comes from fishing and hunting products Characterist cally periods of food abundance alternate with scarcity and sem starvation is not altogether unknown during the winter when transport of suppl es from other d stricts of Greenland may be impossible The people are mostly of mixed Eskimo-Dan sh origin the number of full blooded Eskimos is d ff cult to assess but s bel eved to be about 30 per cent (5)

III *The Angmagssal k area* includ ng the ma n to n Angmagssal k This large area s tuated at the east coast of Greenland between Lat 63 and 66 N is inhab ted by approx 2 200 people living in 10 villages and settlements bes des Angmagssal k (700 nhab) The main occupat on is st ll seal hunting but fish ng is becom ng more and more common In some areas the fish and meat d et s supplemented by imported farm products The people are st ll almost exclus vely pure blooded Eskimos

Al ogether the total populat on of 24 villages or settlements were stud ed A survey team compris ng f ve workers succes-s ely v s ted all illages in the selected areas sett ng up survey cl n cs usually in the local schools Diabetes detect on was only a part of the survey programme which also included the collect on of data relat ng to the prevalence of heart d seases Deta ls about the la ter part of the programme w ll be published elsewhere

In t ally an attempt was made to obta n a short history from each adult concern ng personal and fam ly h story of diabetes but this had to be abandoned due to language d ff cult es s nce the Esk mo language does not conta n words adequate to the concept of d abetes

Urine sample bottles marked w th each nd idual s name were distributed to each household together h a printed instruc t on urging each nd idual to produce a urine sample two hours after the ma n meal of the day This was also stressed in an oral instruct on when the bottles were distributed

A few hours later the bottles were collected or del ered to the survey clinic Urine

samples were tested qualitatively by means of Clinistix

In the case of babies from whom it was considered impossible to obtain a urine specimen the mothers were instructed to press the end of a Clinistix between two folds of a wet nappy and return the Clinistix with the other urine specimens

All the specimens giving a positive reaction with Clinistix (development of a blue colour within two minutes) were tested with Clinitest Five drops of urine were mixed with 0 5 ml of water in a test tube and a Clinitest reagent tablet added The resulting colour was recorded as follows negative trace 1/2 % 3/4 % 1 % 2 % according to the instruction accompanying the Clinitest bottles

Those individuals who showed a trace or positive reaction to "Clinitest were offered a glucose tolerance curve or a fasting blood sugar

Performance of the latter part of the programme was prevented by technical considerations during the first visit of the survey team but was usually accomplished during a later visit of one of the team members to the village or when the individual suspected of diabetes visited the main town of his district

Venous blood samples were obtained from the cubital fossa and kept cool in tubes with dried heparin fluoride until centrifugation (within 24 hours) The plasma was separated and shipped deep-frozen (— 20 C) to a central laboratory in Copenhagen (Medicinsk Laboratorium) The plasma samples were analysed for glucose according to the glucose oxidase method of Råbo and Terkildsen (12)

Hospital statistics

The health service of Greenland is organized in 17 medical districts along the coast each of which (with one exception) is provided with a smaller or larger hospital In addition a central hospital with a medical department is located in the capital Godthaab All medical officers of these hospitals are trained in Denmark

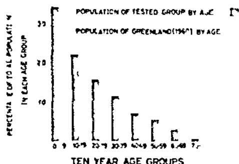


Fig 2 Age distribution of the native population of Greenland and the population tested

The hospital records of all these hospitals have been reviewed with the purpose of listing all known living cases of diabetes mellitus in the native population of Greenland Relevant information as regards sex age duration of diabetes eye ground changes treatment etc has been extracted from the records In 1962 there were 9 347 admissions to these hospitals (total population approx 35 000) Routinely all cases admitted are checked for glucosuria and if present a fasting blood sugar or a glucose tolerance curve is obtained

Results

Population survey

Participation The total population of the three areas chosen for the survey was 4,384 This corresponds to 14 0 per cent of the native population of Greenland and about 7 % of all Eskimos of the world Of these 4 249 or 96 9 per cent were tested Of the 135 non participants 92 or 2 1 per cent of the population chosen for survey were below the age of 3 years

The age distribution of the participants compared with that of the native population of Greenland as a whole is represented graphically in fig 2 It

TABLE I Age sex and geographic distribution of tested population and individuals showing glyco-

Age group	0–9		10–19		20–29		30–39	
	No	+ glyc uria	No	+ glyc uria	No	+ glyc uria	No	+ glyc uria
Godthaab								
Men	199	1	113	0	56	2	64	3
Women	177	0	112	1	53	1	71	1
Umanak								
Men	202	0	118	0	88	5	66	1
Women	192	0	107	0	72	0	65	0
Angmagssalik								
Men	336	0	229	0	182	1	108	0
Women	357	0	257	0	212	0	118	0
Total								
Men	737	1	460	0	326	8	238	4
Women	726	0	476	1	337	1	254	1
Both sexes	1 463	1	936	1	663	9	492	5

appears that the age distribution of the total tested group closely corresponded to that of the native population The same was true when each of the three sub groups were compared with the total population The sex distribution within each group also corresponded closely to that of the population as a whole

The age and sex distribution of the participants in each sub group together with the number of glycosurics found are indicated in table I

The table shows that 0 96 per cent of the men and 0 19 per cent of the women or 0 56 per cent of the test group as a whole manifested glycosuria under the conditions of the test Of the 24 glyco surics only two had 0 5 % glucose in the urine or more

Although the prevalence of glycosuria is generally low it is strikingly higher amongst men than amongst women No definite age trend is apparent

Only two of the 24 glycosurics were found in the pure blooded Angmagssalik population, although this contributed 48 5 per cent of the total group tested

Table II shows the results of the blood sugar studies In 6 of the 24 glycosurics blood sugar was not obtained This was due to technical difficulties rather than lack of cooperation In two of the 6, repeat urine studies were negative for glucose, in one case a trace of glucose was found The remaining three were not tested further These three had shown trace 1/2 % and 1/2 % glucose respectively on the initial survey

suria

40–49		50–59		60–69		70–		Total		
No	+ glycosuria	No	+ glycosuria	No	+ glycosuria	No	+ glycosuria	No	+ glycosuria	%
50	1	29	1	11	1	4	0	576	9	1
58	0	35	1	16	0	6	0	578	4	0
37	1	50	1	29	1	4	0	594	9	1 5
30	0	44	0	24	0	10	0	544	0	0
61	1	37	0	15	0	3	0	[illegible]	2	0
67	0	45	0	25	0	5	0	1 086	0	0
148	3	116	2	55	2	11	0	2 091	20	0 [illegible]
155	0	124	1	65	0	21	0	2 158	4	0 1
303	3	240	3	120	2	32	0	4 249	24	0 [illegible]

TABLE II Blood sugar studies in 24 individuals showing glycosuria

Age groups (years)	No of glycosurics	Blood sugar not obtained			Blood sugar obtained		Diagnoses	
		Repeat urine test Neg	Repeat urine test Pos	Not tested further	Fasting blood sugar	Glucose tolerance test	Diabetes mellitus possible	Diabetes mellitus diagnosed
0–9	1				1			
10–19	1	1			0			
20–29	9		1	1	6	1		
30–39	5			1	3	1	1	
40–49	3				2	1	1	
50–59	3	1		1	0	1		1
60–69	2				2			
70–	0				0			

In 18 of the 24 glycosurics fasting blood sugar was determined In 15 instances the fasting blood sugar was ⩽ 100 mg per cent In three out of these a subsequent glucose tolerance curve was normal (peak blood glucose level less than 180 mg per cent, return to initial value in two hours or less)

TABLE III Documented cases of diabetes mellitus in Greenland

District	Case no	Sex	Age (yrs)	Maximal recorded FBS (mg %)	Episodes of acetonuria	Age at onset of symptoms	Age at diagnosis (—symptoms)	Diabetic eye ground changes	Treatment	Remarks
Egedes minde	1	♀	63	273	0	50		0 (1959)	Insulin	
	2	♂	58	256	?	36		0 (1963)	Sulfonylurea	
	3	♀	52	191	?		46	?	Sulfonylurea	
	4	♀	44	414	+	31		+ (1960) — (?) (1963)	Insulin — cortisone — thyroid extract	Diabetes mellitus — Simmond's disease
Holsteins borg	5	♂	23	154	0		18	?	Diet	
Sukker toppen	6	♂	58	280	+	55		?	Sulfonylurea	Insulin treatment not possible
Godthaab	7	♀	51	190	+		48	?	Diet	Found at survey
Juliane haab	8	♂	23	106	0		19	?	0	
	9	♀	45	312	?	32		?	Insulin	
	10	♀	67	>400	0	63		?	Sulfonylurea	

Of the remaining three two subjects — a man aged 40 years from Umanak district with a fasting blood sugar of 129 mg % and a man aged 32 years from Godthaab district with a fasting blood sugar of 125 mg % — were diagnosed as possible diabetics We hesitated to make a definite diagnosis, since only a single blood sugar determination was available

One single case a woman aged 51 years from Godthaab district, was found to have frank, although asymptomatic diabetes This was diagnosed on the basis of a fasting blood sugar value of 190 mg % and a glucose tolerance curve with the following values 30 minutes 261 mg % 60 minutes 327 mg %, 90 minutes 280 mg % 120 minutes 257 mg % and 180 minutes 206 mg % The urine persistently contained glucose

If all three cases are accepted as diabetics and if it can be assumed that the population sample studied is representative of the native population of Greenland, the total number of diabetics

in Greenland would be about 20 and the prevalence rate 0.6 per mille.

Hospital statistics

The survey of the hospital records from 18 hospitals of Greenland revealed ten known living cases of diabetes *mellitus, five men and five women, among* the native population af Greenland (32,249 in 1962). Some data concerning these cases are listed in table III.

In 12 of the 17 medical districts, with a total population of approx. 17,000, diabetes had never been diagnosed. These included the districts of Thule and Angmagssalik, which are characterized by their almost pure blooded Eskimo population. The absence of diabetes in the district of Angmagssalik was confirmed in the population survey reported above.

Age at onset of diabetic symptoms, or age at the time of diagnosis when no symptoms were present, varied from 18 to 63 years. In six instances the diagnosis was made following a period of typical diabetic symptoms (cases 1, 2, 4, 6, 9 and 10), while in the remaining four urine testing followed by blood sugar determinations revealed the nature of the disease. One of the latter (case 7) was diagnosed during the survey reported above and subsequently hospitalized. The two cases classified in the survey as possible diabetics are not included. In case 8 the diagnosis is based upon an abnormal glucose tolerance curve.

Cases 1, 2 and 3 are siblings. They are of mixed Eskimo-Danish descent.

Only three of the patients are receiving insulin, although in at least one instance (case 6) insulin treatment appears indicated. Four patients receive oral antidiabetic treatment while three get along on diet alone or no treatment at all.

Although episodes of acetonuria have been noted in three instances, a true *case of diabetic coma has never been* recorded in Greenland.

None of the diabetics are adipose.

One of the patients (case 4), described in detail elsewhere (13), presented the rare combination of diabetes and Simmond's disease. She is under treatment with insulin, cortisone and thyroid extract.

A comparison of the hospital statistics with the results of the population survey suggests that for each diabetic known in Greenland there is one hitherto undiscovered case. This is in agreement with the findings in other studies of the prevalence of diabetes mellitus (16).

Discussion

The importance of mass screening programs for the detection of diabetes mellitus has become increasingly evident during the last twenty years and a large number of reports dealing with the prevalence of diabetes mellitus in different communities were recently reviewed by the W.H.O. expert committee on diabetes mellitus (15).

Evidently, a major object of such screening programs is the early detection of cases of unrecognized diabetes mellitus, thereby making possible the prompt institution of adequate therapy.

Another important object, however, is — through the application of epidemiological techniques to population stu-

dies in various parts of the world — to attempt to define the effects of such factors as age, sex, climate, exercise, diet, economic development and race upon glucose tolerance.

A prerequisite for a valid comparison of the results of such studies is the application of uniform test methods and the universal acceptance of standard criteria for the diagnosis of abnormalities of glucose metabolism.

A perusal of the reports published to date reveals that a large variety of test procedures, chemical methods and diagnostic criteria have been employed, and indicates that standardization of the technique for diabetes screening and agreement upon criteria for the diagnosis of diabetes mellitus are urgently needed.

Since the diagnosis of diabetes mellitus depends upon demonstration of hyperglycemia, the most valid procedure in mass detection programmes includes testing of blood specimens from all participants, preferably after a standardized test meal. However, cost consideration and lack of availability of clinical laboratories of sufficient capacity to perform blood glucose tests may force the epidemiologist to decide against this procedure and content himself with the screening for glycosuria alone or, in suspected cases, together with blood glucose studies.

The present study is a case in point. While the testing of urines, utilizing the glucose-oxidase test stick method, was a fairly easy task during a field study in Greenland, the collection of thousands of blood samples in 24 villages distributed over large areas, the centrifuging, deep freezing and transportation of the samples with sledges, small vessels and aeroplane over thousands of miles to reach the clinical laboratory in Copenhagen, simply was not feasible.

As a consequence of this compromise, the basis for recognition of diabetes was simply the presence of postprandial glycosuria. Since the incidence of glycosuria clearly depends upon the carbohydrate load consumed during the meal preceding the testing of urine, dietary habits in some parts of Greenland favoring a high fat, high protein diet might partially be responsible for the low incidence of glycosuria in these parts (villages in the Umanak and Angmagssalik areas), but in large areas covered by the survey (Godthaab area, town of Angmagssalik), the habitual diet does not differ greatly from that consumed in Denmark proper, and in these parts, glycosuria was no more prevalent.

We believe therefore that in spite of the technical shortcomings of the methods employed in this study, the data clearly demonstrate that diabetes mellitus is rare indeed in the Eskimo race. The results of the survey of hospital records support this view.

The explanation of the infrequency of diabetes mellitus among Eskimos is far from clear. It is now generally recognized that diabetes mellitus is a hereditary condition, the clinical appearance of which is influenced by a variety of factors such as age, sex, diet, exercise, body weight and pregnancy.

The *age distribution* of the population surveyed corresponds closely to that of Greenland as a whole. It appears from fig. 1 that not less than 56.4 % of the

Eskimo population of Greenland is below the age of twenty years as compared to 33.5 % in Denmark proper. Since the prevalence of glycosuria and diabetes mellitus increases with age, part of the rarity of glycosuria and diabetes can indubitably be ascribed to this age distribution. The study included, however, a sufficient number of participants over the age of 30 years (1,187) to demonstrate that glycosuria is also rare in these age groups.

It is generally recognized that while the *sex distribution* of diabetes mellitus in the younger age groups tends to be about equal, the prevalence among women after the menopause is considerably higher than among men in the same age groups in most studies. One of the striking features of the present study is the rarity of glycosuria among women in all age groups. It should be noted, however, that the survey of hospital records revealed an equal number of female and male diabetics in Greenland.

As mentioned previously, *dietary* habits vary considerably in different areas of Greenland, being largely dependent upon occupation (fishermen or hunters), economic level and accessibility of imported food. In some areas covered by the survey (e.g. Godthaab area) the dietary composition does not differ greatly from an average Danish diet, while in others (parts of Umanak and Angmagssalik districts) the ancient primitive diet with its relatively large content of protein and fat still prevails. The relative importance of various dietary factors in disclosing the presence of diabetes is still a matter of controversy.

The present study, revealing a low incidence of glycosuria — particularly among women — in areas characterized by widely different dietary patterns, does not contribute towards a solution of this problem.

Since it is well known that physical *exercise* improves glucose tolerance (2) it is conceivable that hard physical labor may have a protective influence against diabetes mellitus. Strenuous physical labor is still in many parts of Greenland a condition for acquisition of adequate food supplies and this might partly explain the relative absence of glycosuria in men — but not in women.

Scott and Griffith (14) showed that Alaskan Eskimos are on the average overweight by white standards, and that *obesity* is by no means uncommon. By contrast, the experience of the present authors in about 4,000 measurements of height and weight in Greenland Eskimos (10) shows that obesity is rare among Greenland Eskimos, particularly among men. Lack of obesity as an aetiological factor in the development of diabetes mellitus might therefore contribute to the rarity of diabetes in Greenland.

Some workers believe that the incidence of diabetes in women increases with each increase in *parity* (6). Pyke (11) found that if a woman has born 5 or more children she is 3 1/2 times more likely to develop diabetes than if she had born fewer or no children. In 1961, the birth rate in Greenland was 50.2‰, probably among the highest in the world, families with 8 or more children are common. Against this background, the relative absence of diabetes mellitus among

women is even more striking It is well known that women with diabetes or pre diabetes are prone to give birth to large babies Fog Poulsen (7) found that 2 4 % of newborn boys and 2 0 % of newborn girls in Greenland had birth weight of 4 500 g or higher These figures are in agreement with those reported by Bolton (3) The Eskimo children or their mothers have not been followed up with a view to detecting diabetes

Thus, none of the factors discussed seem to explain fully the rarity of diabetes mellitus in Greenland or the sex difference in prevalence

The problem arises as to whether the rarity of diabetes in Greenland may be a racial characteristic Knowles (9) believes that there is insufficient evidence to demonstrate convincingly that racial differences exist in susceptibility to diabetes The former belief that diabetes mellitus is rare in the Negro race was rejected by Joslin (8) who showed that diabetes mortality among Negroes in the United States was not much lower than that among the white population On the other hand, Joslin believed that diabetes was particularly prevalent among Jews The problem of racial susceptibility or racial resistance to the development of diabetes is complicated *by the fact that the differences in the* reported prevalence rates of the disease in different ethnic groups are almost certainly related mainly to differences in age distribution of the populations, availability of medical care, activity dietary composition and total caloric intake

We believe that the results of the present study however showing that diabetes mellitus occurs infrequently in the Eskimos even in those living under environmental conditions not much different from most western countries, support the concept of a racial resistance to the development of diabetes The fact that not a single case of diabetes or suspected diabetes was found among 2,100 pure blooded Eskimos from the East coast of Greenland, while the single definite case of diabetes, the two possible cases and 92 % of the glycosurics were found among the 2,100 inhabitants of predominantly mixed Danish Eskimo descent on the West coast, lends further support to this concept

If on the other hand, the low prevalence of diabetes mellitus in Greenland is solely related to environmental factors, then the rapid urbanization, industrialization and consequent changes in dietary habits taking place in Greenland after World War II, will lead to an increase in diabetes prevalence in the future

Summary

4,249 Eskimos living in three different areas of Greenland were surveyed with the object of disclosing diabetes mellitus

24 were found to have post prandial glycosuria *In 18 of these a fasting blood* sugar or a standard glucose tolerance test was obtained

One case of unequivocal diabetes mellitus was found two others were classified as possible diabetics

A review of the records from all hospitals in Greenland revealed 10 known cases of diabetes in a native population of approximately 32 000

Possible causes of the rarity of diabetes mellitus among Eskimos are discussed

Acknowledgement

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The Effect of Artificial Ventilation in Tank Respirators on Mixed Venous pCO_2 in Patients with Chronic Pulmonary Failure

By

Helge Grendahl

The purpose of artificial ventilation in patients with pulmonary failure is to increase the minute ventilation as the spontaneous ventilation is insufficient for adequate carbon-dioxide elimination and oxygen uptake

In a respirator with an un restricted air flow (i e a tank respirator) the minute ventilation will at a constant frequency be proportional to the res piratory pressure used when the flow rate and volume changes are not too great (8) The CO_2 diffusion in the lungs is very rapid The CO_2 excretion will therefore be proportional to the minute ventilation and thus to the respiratory pressure *if all parts of the lungs are* evenly ventilated In patients on artificial ventilation however *uneven ventilation* with an increase in functional dead space and inadequate ventilation blood flow relationships may be seen and thus the pulmonary gas exchanges may *not* always be strictly *proportional* to the minute ventilation (4)

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In this investigation the actual effect of artificial ventilation with different respiratory pressures in a tank respirator as expressed by the reduction in mixed venous pCO_2 is observed in patients with carbon dioxide retention due to chronic pulmonary disease

Material and methods

The investigation was performed on four patients with a severly reduced pulmonary function and carbon dioxide retention The main clinical data were

Patient No 1 64 years male In 1935 thoracoplasty for pulmonary tuberculosis In 1961 carbon dioxide narcosis provoked by bronchopneumonia and oxygen therapy successfully treated in a tank respirator The following years periodically artificially ven tilated from 1963 he has been permanently in hospital lying in a tank respirator for 8 hours every night In daytime out of bed with severe exertional dyspnoea Vital capacity 1 300 ml Peak flow 80 l min

Patient No 2 67 years male From 1930 asthmatic bronchitis In May 1964 pneu monia and severe carbon dioxide retention

(arterial pCO_2 95 mm Hg pH 7 17, HbO_2 41 %) relieved by artificial ventilation in tank Later on stayed in hospital lying in the tank for 10—12 hours every night By day out of bed and fairly mobile but periodically disorientated probably due to carbon dioxide retention Vital capacity 1 790 ml Peak flow 100 l/min

Patient No 3 68 years male Chronic bronchitis with emphysema for the last 10—15 years In 1962 acute exacerbation with unconsciousness following oxygen administration (arterial pCO_2 110 mm Hg pH 7 10) successfully ventilated in the tank respirator The following years hospitalized in periods for artificial ventilation Out of bed moderate breathlessness on effort Vital capacity 2 000 ml Peak flow 90 l/min

Patient No 4 54 years male From childhood chronic asthmatic bronchitis In 1963 disorientated with severe carbon dioxide retention (venous tot CO_2 44 mEq/l), successfully treated in tank respirator Later on in periods artificially ventilated Out of bed severe breathlessness on effort Vital capacity 1 290 ml Peak flow 60 l/min

At the time of the investigation the patients were in a clinically stable period They were ventilated in tank respirators for 8—10 hours every night and were well accustomed to the treatment The effect of artificial ventilation for a period of 60 minutes was investigated Mixed venous pCO_2 was measured before and after 60 minutes of continuous ventilation The tests were performed about 12 a m after the patient had been quiet in bed for at least 30 minutes and without artificial ventilation for at least 3 hours The expiratory pressure was + 3 cm of water while the inspiratory pressure in the different tests varied from — 15 to — 35 cm of water The respiratory frequency was 18—22 per minute

Standard Emerson tank respirators were used The maximum negative (intra tank) pressures obtained were — 37 — 30 — 30 and — 28 cm of water respectively in the 4 tanks employed A careful tightening of the neck-collar was necessary when maximal pressure was wanted

Mixed venous pCO_2 was measured using the rebreathing method described by Campbell and Howell (2 3) A mouth piece was used in all the tests reported on here A rubber anaesthethic face mask was first tried but was found unsatisfactory in preventing leaks during the sampling The CO_2 analyses were performed in the simplified Haldane apparatus described by Campbell (1) Double analyses were always done and in 193 double analyses the average difference was 0 6 % (i e a difference in pCO_2 of about 0 4 mm Hg), in 23 of these, where the differences exceeded 0 15 % triple analyses were done

Peak flow was measured in a Wright's Peak Flow Meter Vital capacity was measured by an Emerson breathmeter with the patient lying in bed

Results

In the test period (i e about 2 weeks) the resting mixed venous pCO_2 was fairly stable in each patient The effect of artificial ventilation in tank respirators with varied negative (inspiratory) pressures on patients with chronic lung failure is shown in table I pCO_2 was falling in all tests except 2 (in pts 2 and 3) where a pressure of — 15 cm, of water did not induce any reduction The best and most constant effect was seen in the patient with pulmonary failure after thoracoplasty (pat No 1) In the other patients, suffering from chronic bronchitis, the effect of artificial ventilation was less pronounced A considerable variation was seen especially in pat No 2 where incomplete relaxation in the respirator and a varying degree of asthma on different days probably influenced the results

TABLE I The effect of artificial ventilation in tank respirator on mixed venous pCO_2 in patients with chronic pulmonary disease

		Mixed venous pCO_2 (mm Hg)		Reduction in pCO_2 after artificial ventilation	
	Inspiratory pressure	Before	After		
Patient no	(cm of water)	60 min of artificial ventilation		(mm Hg)	(% of original value)
1	15	57.4	55.5	1.9	3
	15	60.6	55.7	4.9	8
	22	56.9	50.6	6.3	11
	22	56.8	52.2	4.6	8
	22	61.0	55.3	5.7	9
	22	59.0	51.4	7.6	13
	22	57.6	[illegible]4.6	3.0	5
	30	59.6	48.6	11.0	18
	30	58.9	50.5	8.4	14
	30	60.6	47.4	13.2	22
	30	62.4	51.0	11.4	18
	30	54.8	48.2	6.6	12
	35	60.2	47.9	12.3	20
	35	58.2	46.0	12.2	21
2	15	57.6	52.1	5.5	9
	15	60.7	58.7	2.0	3
	15	72.7	73.2	+0.5	−0.7
	22	61.6	53.9	7.7	12
	22	50.8	46.6	4.2	8
	27	61.6	50.2	11.4	19
	29	57.2	51.7	5.9	10
	30	56.3	43.0	13.3	24
	30	53.2	47.3	5.9	11
	33	57.0	46.5	10.5	18
	35	58.0	50.4	7.6	13
3	15	65.6	66.2	+0.6	−1
	15	64.5	59.6	4.9	8
	22	68.9	63.5	5.4	8
	22	69.5	67.4	2.1	3
	22	60.9	59.7	1.2	2
	28	67.4	60.4	7.0	10
	29	69.9	58.9	11.0	16
4	15	70.4	69.0	1.4	2
	15	71.4	64.3	7.2	10
	22	72.5	68.0	4.5	6
	22	69.7	64.8	4.9	7
	27	72.5	63.8	8.7	11
	27	76.0	69.2	6.8	9
	30	71.9	64.2	7.7	11

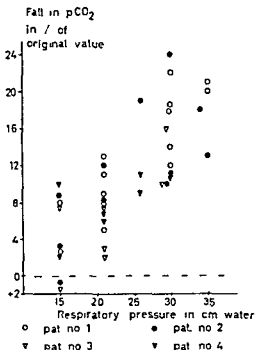


Fig 1 Reduction in mixed venous pCO_2 after 60 minutes artificial ventilation in a tank respirator Relationship to the respirator pressures used

The relationship between the fall in pCO_2 (expressed as a percentage of the original level) and the inspiratory pressure used, is shown in fig 1 A near linear rise in the effect of the artificial ventilation is seen, as the inspiratory pressures increase In the 4 patients examined the average reduction in mixed venous pCO_2 was 4 6 % when an inspiratory pressure of 15 cm of water was used and 14 % at an inspiratory pressure of 27—30 cm

Comments

The main purpose of artificial ventilation in patients with chronic lung failure is to reduce the carbon dioxide retention In a patient lying in a tank respirator this effect can, in our experience, be adequately followed by repeated examinations of the mixed venous pCO_2 by the rebreathing method of Campbell and Howell This method is fairly rapid, repeated arterial punctures or in dwelling canulas are avoided, and the laboratory equipment is not expensive The accuracy of the method is reported to be satisfactory for its clinical use (5,7) Campbell and Howell have estimated it to be within +/— 3 mm Hg or possibly better (3)

In this investigation were the differences between two tests at 60 min intervals were measured, some scattering of the results was noted This may be caused partly by a summing up effect of the observation failures, and be partly due to the fact that the effect of artificial ventilation in a patient, even when in a clinically stable state, is not necessarily identical on different days

This investigation on the effect of artificial ventilation in tank respirators in 4 patients with lung failure in a clinically stable period, has demonstrated that even a moderate negative inspiratory pressure will usually induce a fall in pCO_2 but a considerably greater effect will be seen when higher respiratory pressures are used

The results indicate that in a patient with severe CO_2 retention pressures near maximum for this type of respirator will be necessary in order to get an adequate CO_2 reduction Clinical experience, however (6), has shown that ventilation of patients with severe CO_2 retention with pressures of — 20 to — 22

cm of water have been successful in the majority of cases even though the CO_2 reduction induced by artificial ventilation in tank respirators at these pressures is moderate. This is probably partly due to an increased ventilation and partly because the patients are relieved of the respiratory work which may be great in persons with chronic lung disease.

Summary

In 4 patients with chronic lung failure and CO_2 retention the effect of artificial ventilation in a tank respirator on mixed venous pCO_2 is measured when different inspiratory pressures are used. A considerable rise in the effect of artificial ventilation was noted when higher negative inspiratory pressures were used.

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Pregnancy and Chronic Myeloid Leukaemia

Follow up Study of Children and Grandchildren

By

A Bjure[1]

In 1956 the importance of following up the children of leukaemic mothers was strongly emphasized by Biermann et al (6) The authors express the need for continuous follow up examination of such children having regard to the conceivability according to them, of hereditary factors in this connexion No such case had up to that time been found however

After a lecture by Ask Upmark on leukaemia and pregnancy in 1957 I gave a brief account of the course of the leukaemia in a patient Mrs M G and also described the clinical findings in her then 26-year old daughter Mrs S I (unpublished)

Six years later (in 1963) I again contacted Mrs S I and made a further report about her in connexion with a second lecture by Ask Upmark on a follow up study of children of leukaemic mothers This and his detailed review on Leukaemia and Pregnancy published in 1961 (3) prompted me to collect as much new information as possible concerning both the mother s illness and the condition of her now 32 year-old daughter from the time of birth

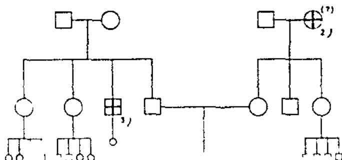
The account that follows embraces the leukaemic mother and above mentioned daughter another daughter, Mrs F M one and a half year older and two children to each of the daughters

As can be seen from fig 1 there had been a relatively high incidence of (confirmed) cancer — three cases of carcinoma of the stomach (in two sibs and an uncle) one of carcinoma mammae (in a sister) and one of carcinoma of the uterus (in a niece)

Before the year 1900 in Sweden the cause of death was unfortunately not certified by the medical practitioner and it is therefore impossible to follow the family tree farther back than this On the death certificate however there is a note that the patient s maternal grand mother died of an abdominal tumour which may have meant cancer ventriculi an assumption not contradicted by a grandson s (my patient's

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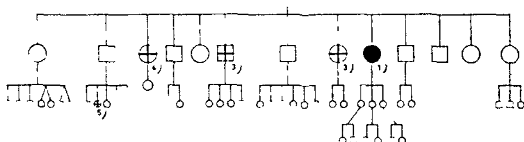


Fig 1 ☐ male ○ female Family tree 1) Chronic myeloid leukaemia 2) "Abdominal tumour 3) Cancer ventriculi 4) Cancer mammae 5) Cancer uteri

TABLE I Mrs M C b 28 3 1904

Date	% Hb	Cell count per mm: Red cells	White cells	Thrombocytes	Diff count (%): Megaloblasts[1]	Normoblasts[1]	Myeloblasts	Promyelocytes
1 23/10 30	63	3 140 000	125 000		0	7	0	0
2 1/11 30		–	167 000		0	1	0	5
3 20/2/31	85	3 810 000	7 200	231 000	0	1	0	0
4 4/4/31	90	3 710 000	42 600		1	0	0 5	3
5 29/11/31	88	3 770 000	72 000	357 000	1	2	0 25	10
6 7/11/32	64	2 780 000	6 600	–	0	5	0	7

[1] Per 400 white cells counted in Nos 1 2 4 5 per 200 in Nos 3 6

cou n) descript on of th s woman s ca e

It is rotewortl y tl at tl e u cidence of cancer is clearly greater among tl e fam lies of pat ents v ith leukaem a tl an in control series (17 27) It is al o of in terest to note tl at Crafl et al (1) s c ceded in provoking leukaemia by injecting of cell free tumour f ltrates in m ice

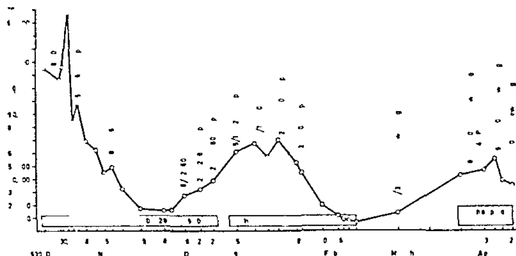
Case reports

M s M C III para b 28 3 1904 d 11 1 1933 of chron c myelo d leukaem a Sl e ha l been in largel good heal h u nt l sl e developed le kaem a At tl e age of abo t 10—12 years she had l ad eacl a tumn sl gl t jo nt trouble affect ng t e hands and feet In 1926 she ha l l ad so ne stomach t ouble of ulcer type b t X ray d sclosed no ulcer Her first del very 7/7 1928 was normal During the two months preced ng her second del ery 3 10 1929 her general heal h was so im pa red tl at her husband s spected sl e had tuberculos s Desp e extreme lass tude she refused o go to tl e doctor s nce sl e l ad no pa n Sl e almost bled to death o ing to fa lure of separat on of the placen a wh ch was del ered by the m dw fe af er 5 ho rs No blood test was taken

Her cond t on grad ally impro ed an l she rema ned fa rly well unt l spring 1930 In tl e m ldle of March sl e not ced tl at tl e left s de of the abdomen felt l ar ler than tl e r gl t and her husband v as al le to palpate a har l non tender m a s extend n abo t a hand l rea l h below tl e left costal margin During tl e spr ng sl e a a n began to feel t red and exl a sted B m l mer she ha l been tw ce to tl e doctor b t l er l sl an l cannot remem ber whether or not sl e men t oned the splen c enlargem ent at tl at t me Iron and a ton c were prescr bed b t n blood tests were m ade Altl o h sl e became progress ely worse sh ld not go back to tl e doctor 30 10 S e a then referred to tl e departm ent f med cine Central H sp al Vasterås under tl e d a nos s of pregnancy splen egal and was a lm t ted tl ree da s later on 22 10 1930

On adm ss on tl e pat ent as ery poorly She was pale and we gl ed 45 7 kg he ht 157 cm Heart s ze normal s s ol c m urm ur lo dest over the ba e Blood pressu e 05 55 mm Hg Abdom en the left s de was f lled by the grea ly enlarged spleen wh cl exten le l several cm l elow tl e level of tl e l ac crest to w th n l 2 cm of the m dl ne The brea th o

Myelocytes			Meta myelo cytes	Polymorphs			Mono cytes	Lympho cytes
Neu tro phil	Eos no phil	Baso phil		Neu tro phil	Eos no phil	Baso phil		
4 2		0	7 5	57 5	0 75	0 5	0	3 75
1	0	0 5	15	58	0	1	1 5	1
2	0	[illegible]	0	61	0	1	[illegible]	30
17	0	0	5 5	51 5	1	1	6	14
5	0 5	1	7 5	47 25	0 5	2	1 7	4
7 5	0	0	1	48	0	4	14	18 5



I g 2 Wl e-cell count in case Mrs M C

the spleen was 11 cm and the free border was 14 cm f om the costal marg n The organ was frm and non tender The l ver was not palpable The uterus was about 10 cm long the s ze of a coconut (last menstr al per od 15th — 0th July Urine traces of albun n sed ment numerous leukocytes and a fey ep th el al cells Blood Hb 63 % 10 6 g per 100 n l accord ng to Enghoff s Standard red-cell count 3 14 m ll wh te-cell count 125 000 mm³ The d fferent al wh e-cell co nt sl owed that the pat ent as suffering from cl ron c n yelo l leukaem a tal le I

Ow n to the pat ent s poor cond t on e were doul ful if it was w se to let the preg nancy cont nue furthermore bo h the pa t ent *and her husband begged to ha e it* term nated Af er consul at on w th an ob- stetr cian we dec ded not to term nate the pregnancy s nce it seemed l kely that the pat ent s cond t on could be cons derabl improved by rad otherapy thus enabl ng her to l ave ano her vial le cl ld

Ow ng to the great risk of damage to the foetus if the uterus is rrad ated 28 we were extremely caut ous from the f rst and only the upper lateral pa t of the spleen was trea ed The rest of the body was ent rely covered th lead sh elds Later these be came unnecessary o ng to the development of roentgen t bes w th b lt n sh elds)

Irrad at on of the spleen was started on the s xth day after adm ss on to l osp tal Iron an l arsen c were also given Cons derable im provement soon took place n the pa ent s general cond t on The spleen became pro- gress el smaller the haemoglol n value rose and the l te-cell count fell see tal le I and f g 2

Up to the t me of del ery 11 4 1931 the pat ent rece ed 11 treatments on the spleen the last one 2 2 1931 a total f 900 r Af er the lat er date she rece ed treatmen s *of 150 r each on the legs on 11 3 and 8 4* respect el see f g

Thanks to the r goro s safety meas res we felt fa rl conf dent that the foet s co ll l a e s s a ned no rad at o njury e en tho gl therapy was s ed just af er the f rst trimester dur ng wl l per od the risk is greatest

Concern ng the course of the llness n other respects, the follow ng may be of interest At the end of 1930 and the beg nn ng of 1931

TABLE II Mrs S I b 11/4/1931

	°	Cell count per mm				Diff count of 200 white cells (%)				
						Polymorphs				
Date	Hb	Red cells	White cells	Poly morphs	Mono-nuclears	Neutrophil	Eosinophil	Basophil	Monocytes	Lymphocytes
15/4/31	—	—	7 200	2 800	4 400		—	—		
2/9/31	107	4 580 000	13 400			16 5	0 5	0 5	1 5	81
2/12/31	85	3 850 000	10 100			31	0 5	0	4 5	64
26/3/47	58	2 700 000	7 300		—	62	2	2	6	28
7/5/47	87	3 950 000								

considerable bruising took place The thrombocyte count was unfortunately not done until 20/2/1931 when it was 231 000 At about the middle of February the patient developed fairly severe alveolar periostitis and stomatitis with deposits on the gums and buccal mucous membranes After operation this cleared up within a week

As can be seen from table I and fig 2 the *white-cell count had fallen to normal 7 200* by 20/2/1931 The patient s general condition was much improved as was also her capacity for work which at this time could be described as fairly good She was therefore discharged on 23/2/1931 with instructions to report at regular intervals

After returning home the white-cell count again began to increase and the two irradiation *treatments of the legs* were therefore undertaken

Normal delivery took place on 11/4/1931 The infant a girl weighed 3 000 g There was normal blood loss and the placenta was delivered after 5 min

The mother remained under careful observation Thanks to repeated radiotherapy chiefly of the spleen it was possible to keep *the white cell count fairly low until autumn* 1932 Throughout this time the patient s capacity for work was more or less sufficient for being able to run her home

The spleen continued to diminish until 30 3/1932 when it extended only 2 cm below the costal margin The liver on the other hand which could not be palpated at the time of admission had gradually increased in size and on 30 3 1932 was felt about 4 cm below the costal margin

Owing to the patient s relatively good health and the resumption of menstruation we kept in mind the possibility of a new pregnancy despite the lowered fertility in leukaemia

In 1929 Neumann published a review of the known cases of pregnancy in leukaemia 30 in all and 15 of them with chronic myeloid leukaemia According to Neumann a full term viable first infant was to be expected in this form of leukaemia provided conception took place shortly after the onset of the illness If on the other hand the first pregnancy occurred during a later stage of the illness or if the pregnancy was not the first one Neumann was of the opinion that it should be terminated without delay

In my case the question was whether to wait and to terminate a new pregnancy if it should occur or to advise sterilization Since *another pregnancy might well hasten the* patient s death it was decided after consultation with the same obstetrician to have her sterilized and this was done on 31/3/1932 at

TABLE III. Two daughters and four grandchildren to Mrs. M. C.

Name and date	Weight (kg)	Height (cm)	Hb (%)	Red cells	White cells
Mrs. S. I.					
b. 11 4 31 24 9 57			71	3 900 000	6 500
19 9 63	55	163 5	79	4 480 000	10 500
R. I.					
b. 23 4 55 19 9 63	25	127	72	–	6 700
C. I.					
b. 2 2 58 19 9 63	18 5	110	77	–	8 600
Mrs. E. M.					
b. 3 10 24 17 11 63	6[illegible]	1[illegible]9	75	4 040 000	7 600
T. M.					
b. 20 7 51 17 11 63	36	159 5	90	–	6 400
A. K. M.					
b. 24 2 53 17 11 63	27	131	84		8 100

the department of surgery. Healing was delayed by the development of a large haematoma in the wound and the patient could not be discharged until 25 4 1932 when she was in fairly good condition.

Towards autumn 1932 the patient deteriorated. There was a marked increase in the white-cell count (149,200 on 12 10) and gradual increase in the size of the spleen which on the same date extended 2 cm below the level of the iliac crest and slightly beyond the midline. The liver had also increased in size. The patient was therefore re-admitted to the department of medicine for a new course of radiotherapy which it was hoped would yield better results than the previous outpatient treatment. For a few months this was the case. The white-cell count fell to fairly normal values, but the anaemia unfortunately increased rapidly. During the fourteen days before death on 11 1 1933 the white-cell count rose sharply to a final figure of 640 000 mm³.

Autopsy 12 1 1933. Liver greatly enlarged (3.2 kg). Spleen also much enlarged (2.2 kg). Numerous myeloid cells present in liver, spleen and kidneys; oxidase reaction positive.

Diagnosis: myeloid leukaemia.

Follow-up study of two daughters and four grandchildren to Mrs. M. C.

Mrs. S. I., b. 11 4 1931 in vertex presentation. Birth weight 3 000 g, length 47 5 cm, circumference of skull 32 cm. She was breast fed for 5 months and developed normally according to case notes dated 2 9 (weight 5 620 g) and 2 12 1931 (reaches after objects, teeth will soon appear in the lower jaw). Her blood values during 1931 are given in table II.

We then lost touch with her until 1956 when she was treated as an outpatient for anaemia that proved rather refractory. In February she developed symptoms of gastritis and recurrent diarrhoea. At about the same time she developed a recurrent nerve paresis from which she recovered completely after about 4 months.

Thrombocytes	Diff count of 200 white cells ( )				
	Polymorphs				
	Neutrophil	Eosinophil	Basophil	Myelocytes	Lymphocytes
	5	0	1		1
165 000	87	0	0 5	4	1
	38	0 5	0 5	8	53
	46	4	1	4 5	44
138 000	56		0 5	9	37
	49	4	0		42
	66	3	0 5	3	

She was admitted to the department of medicine on 24 3 1947 where she remained for three weeks under the diagnosis of anaemia achylia gastrica She was discharged greatly improved after treatment with special diet hydrochloric acid iron and liver preparations The haemoglobin rose from 58 % on 26 3 to 82 % on 7 5 and the red-cell count rose from 2 76 m ll to 3 95 m ll during the same time The white-cell count was normal and no pathological forms were present (table II) Sternal puncture carried out in March was normal While in hospital the patient had no diarrhoea X ray examination of the stomach and colon showed nothing abnormal The patient was followed up regularly by us until 21 6 1950

In connexion with varicella in June 1947 a number of refractory ulcers appeared on the neck elbows and legs and healed only after several weeks in patient treatment at the surgical department

In autumn 1947 the diarrhoea returned It was more severe than in the previous spring and continued for several weeks In November she was operated on for a cervical lymph node abscess

When I again saw this patient in 1955 she told me that since 1950 she had frequently had sore throat and quinsy In connexion with one of these attacks in 1952 she developed swelling of the finger and knee joints from which she recovered after two months treatment at a special rheumatism hospital in 1954 Gold therapy was not given She again developed arthralgia in 1956 but since then has had no trouble

According to case notes made at the Caroline Hospital Stockholm the diarrhoea which had recurred in mild form several times during the years 1947—1950 became successively worse from 1950 with two or three loose slimy stools per day at that time increasing steadily in number and wateriness In 1953 X ray investigation had disclosed colitis Treatment with diet azulfidine Salazopyrin and phthalylsulphathiazole Sulftalyl produced improvement and the patient remained fairly well on this regime until summer 1956 when the colitis became

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19/9/63	56	163 5	79	4 480 000	10 500
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(b 23 4 55) 19 9 63	25	127	72		6 700
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(b 2 2 58) 19 9 63	18 5	110	77		8 600
Mrs E M					
(b 3 10 29) 17 11 63	6	159	75	4 040 000	7 600
T M ♂					
(b 20 7 51) 17 11 63	36	150 5	90		6 400
A K M					
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Necropsy (12/1/1933) Liver greatly enlarged (3 2 kg) Spleen also much enlarged (2 2 kg) Numerous myeloid cells present in liver spleen and kidneys (oxidase reaction positive

Diagnosis myeloid leukaemia

Follow-up study of two daughters and four grandchildren to Mrs M C

Mrs S J b 11 4 1931 in vertex presentation Birth weight 3 000 g length 47 5 cm circumference of skull 32 cm She was breast fed for 5 months and developed normally according to case notes dated 2/9 (weight 5 820 g and 2 12 1931 reaches after objects teeth will soon appear in the lower jaw) Her blood values during 1931 are given in table II

We then lost touch with her until 1946 when she was treated as an outpatient for anaemia that *proved rather refractory* In February she developed symptoms of gastritis and recurrent diarrhoea At about the same time she developed a recurrent nerve paresis from which she recovered completely after about 4 months

The four grandchildren to the leukaemic mother R I C I T M and A K M were all healthy Their blood values are given in table III

Discussion

In retrospect we can now say that the diagnosis in this case (Mrs M G) was clear in March 1930, when splenomegaly was noted Owing to the often slow and insidious course of chronic myeloid leukaemia it is never possible to establish when the disease process sets in Hoffman and Craver (20) report a mean of 0 75 years for the duration of symptoms before confirmation of the diagnosis in sixty three cases, Minot et al (23) give a mean of 1 24 years for the interval between the first symptom and diagnosis in sixty eight cases In my case having regard to the husband s observation concerning his wife s extreme tiredness a few months before her second delivery on 3/10/1929 (she had had no such symptoms before her first child was born), which thus took place five months before the splenomegaly was noted it is probable that the leukaemia developed before this birth I am therefore including an account of this child (Mrs F M)

It is worthy of note that there are reports in the literature of two (or three) viable infants born of the same leukaemic mother (see Ask Upmark s review p 651)

Owing to the genetic aspects I have also examined the patient s four grand children

Claims of the transmission of acute leukaemia (25) and chronic myeloid leukaemia (8 9, 21) from mother to infant have been strongly refuted (5, 19 26) Furthermore, a number of clinicians (1, 12, 13, 18), have stressed that the placenta constitutes an impenetrable barrier between the leukaemic mother and her child In 1958, however, Cramblett et al (11) published the case of a previously well girl that died 9 months old of acute lymphatic leukaemia after only a few days illness and whose mother had died of the same condition a few days after delivery To judge from the following quotation the authors believe it probable that the leukaemia was transmitted from mother to infant

The relative rarity of acute lymphatic leukaemia during pregnancy and the infrequency of leukaemia in children of less than a year of age make it unlikely that the case reported above occurred by chance only Against this may be argued that according to Cooke (10) and to Gauld et al (14) acute leukaemia is by no means rare during the first year of life and Gauld et al state that such leukaemia may develop as early as the second month It is therefore probably justifiable to say that to date no definite evidence exists that transmission of leukaemia has ever occurred in man

In animals e g mice there is no difficulty in transferring leukaemia by injecting a cell free filtrate from leukaemic dams (Gross and others) According to Krischke and Graffi (22) such transfer may occur within the uterus in a small proportion of cases These two workers have now (1963) succeeded in showing that transmission of leukaemia virus may take place via the milk In one experimental series the young of healthy dams were suckled by leukaemic dams and

74 % developed the disease. In another series in which the young of leukaemic dams were fed by healthy dams, leukaemia developed in only 5 %. It was also shown that the 'vertical' transmission of the virus of myeloid leukaemia of mice is mainly effected by the mother's milk, and only to a lesser extent by the intrauterine route. The possibility of transmission by the milk factor in man was brought up several years ago by Ask Upmark.

In this connexion it is of particular interest that both children and grandchildren to my leukaemia patient were reared on their own mother's milk for periods ranging from two to nine months. None of them has shown any sign of leukaemia.

Summary

1. The case is presented of a woman with chronic myeloid leukaemia who became pregnant at a relatively early stage of her illness.
2. Thanks to irradiation of the enlarged spleen, exercising the most rigorous precautions, that organ diminished in size and the patient's general condition improved and she became fit for work for a considerable time.
3. The infant was born at term, and her subsequent development was normal. At about 15 years of age she developed anaemia that proved very resistant to treatment.
4. Later, this child showed an initially relatively mild colitis that subsequently developed into ulcerative colitis requiring two major operations at 28 and 29 years of age respectively.
5. The anaemia and ulcerative colitis mentioned in paragraphs 3) and 4) may probably not be ascribed to radiation injury to the foetus.
6. It is probable that the mother had leukaemia during the last two months of her preceding pregnancy, which ended 1 1/2 years before the birth of the above mentioned child. This elder child is at present in good general health.
7. The four grandchildren to the leukaemic woman have also been examined and found healthy.
8. No transmission of the disease has been detected in either children or grandchildren. In discussing this topic regard has also been paid to the milk factor, which in animal experiments has proved capable of taking part in the transmission of leukaemia virus.
9. The investigation is a contribution to the follow up studies of children to leukaemic mothers, the importance of which has been stressed by Biermann et al. (6) and also by Ask Upmark (3).

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Cardial Arrhythmias Treated with Synchronized DC-defibrillation

By

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During the last two years it has been firmly established that different types of cardiac arrhythmias can be successfully treated by a synchronized direct-current shock i e a powerful electrical shock of short duration applied at a well-defined point in the cardiac cycle (4 10 11 13 14, 16, 17, 18, 20, 22, 23)

This treatment has especially been used in patients with atrial fibrillation (including chronic cases) atrial flutter, atrial tachycardia, and paroxysmal tachycardia both supraventricular and ventricular Moreover, in patients with ventricular fibrillation there is evidence that DC defibrillation has advantages over the formerly used AC-defibrillation

In the following we shall present our experiences with this treatment, applied to an unselected group of patients — mostly with *chronic atrial fibrillation* — in whom follow up studies were performed 6 to 15 months after the electrical defibrillation

Material

From October 1963 to July 1964 66 DC-countershocks were given to 55 patients In 9 patients the treatment was repeated from five days to four months later and one patient was treated three times at intervals of six months

The principal purpose of this study has been to contribute to the indications of this new treatment for which reason the material has been as unselected as possible

Table I shows the data for the patients Their ages ranged from 32 to 83 years, and 3/5 were men

The cardiac arrhythmia in most cases was an atrial fibrillation Five patients had an atrial flutter or atrial tachycardia One patient had a long standing attack of supraventricular tachycardia and one had ventricular tachycardia for the past 24 hours as a complication to an acute coronary occlusion

An approximate estimate of the duration of the arrhythmia was obtained in all patients except two However this was a minimum value i e the time from the first demonstration of a permanently irregular pulse In about 1 4 of the patients there was information of paroxysms of arrhythmia several years earlier

Concerning the cardiac diagnosis it is shown in table I that about 2/5 of the patients had no other signs of heart disease than atrial fibrillation in some cases with a slight degree of heart enlargement In 10 patients there were signs of coronary disease and one patient had a seven day-old coronary

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TABLE I. Cardiac arrhythmias treated with synchronized DC-countershock

	No. of patients (No. of treatments)		Still sinus rhythm		
	Total no.	Reversed	At dismissal	At follow-up 2–3 months	At follow-up 6–15 months
Total	55 (66)	44 (53)	32	19	14
Sex: Men	33 (41)	27 (33)	19	14	11
Women	22 (25)	17 (20)	13	5	3
Age: Years 32–49	11 (15)	9 (12)	[illegible]	8	4
50–69	36 (41)	29 (33)	20	9	[illegible]
70–83	8 (10)	[illegible] (8)	3	2	1
Duration of arrhythmia					
< 1 month	9 (13)	9 (12)	[illegible]	4	4
1 month–1 year	19 (2[illegible])	18 (20)	17	11	
1 year–5 years	13 (16)	9 (12)	6	2	1
> 5 years	1[illegible] (13)	6 (7)	2	1	1
Diagnosis					
No other heart disease	20 (24)	18 (21)	13	11	9
Coronary disease	10 (13)	8 (11)	4	2	1
Valvular disease	14 (14)	8 (8)	8	3	1
Hypertensive heart disease	2 (2)	2 (2)	1		
Seq. thoracotomy	2 (3)	2 (3)			
Traumatic heart disease	2	2 (2)	1	1	1
Hyperthyroidism seq.	4	3 (5)	2	2	2
Pulm. cancer	1 (1)	1 (1)			
Cardio-thoracic index					
≤ 50	10 (12)	9 (11)	[illegible]		3
51–60	15 (18)	11 (13)	8	[illegible]	5
61–73	9 (10)	6 (7)	3	1	1

infarction. Fourteen of the patients had valvular lesions: one had aortic stenosis and 13 mitral stenosis. In one of the mitral cases the fibrillation was a postoperative complication. Two patients had symptoms of hypertensive heart disease, one of them with acute pulmonary oedema at admission supposedly provoked by the atrial fibrillation. In two patients the atrial fibrillation appeared after a thoracotomy five months and three years prior to the study, one for a paralysis of the diaphragm with lung atelectasis and the other for a heart tumor. The latter patient subsequently developed a ball thrombus in the left atrium. In two other cases the atrial fibrillation seems to have started in connection with severe trauma of the thorax, in one case 20 years ago in a concentration camp and in the other after an accident two months earlier. Three patients had thyrotoxicosis and one was treated with thyroid for obesity when the atrial fibrillation started. Finally one patient had an advanced inoperable cancer of the lung.

In 34 patients an X-ray of the thorax was taken just before the treatment. Most of the patients had a slight, some a more pronounced, enlargement of the heart assessed from the ratio between the heart shadow and the diameter of the thorax.

Procedure

All patients were given quinidine 500 mg daily from the day before the DC-defibrillation, in some cases as a continuation of long-term treatment. Only those patients who were digitalized — these being in the majority — continued with the normal maintenance doses. All patients were anticoagulated except two, a woman with metrorrhagia and a man with acute hepatitis. Patients with valvular lesions and heart enlargement were on anticoagulants for at least two months prior to conversion, and in the remaining patients the treatment was performed as soon as the P-P value was at therapeutic level.

All patients were anaesthetized with fluothane (Halothanum NFN), N_2O, O_2, but without any premedication. The purpose of the anaesthesia was threefold: to save the patient from the nervousness and discomfort of the electric shock, to avoid any movements of the muscles which might release the impulse prematurely, and to be in the best position to counter complications.

The defibrillator used was a "Cardiac Syncronizer" from Corbin-Farnsworth, and the standard lead with the largest voltage was used to synchronize the countershock to the S wave of the cardiogram. The electrodes having a diameter of 9 cm were placed with the positive one over the lower part of the right sternal border and the indifferent one over the left subscapular region. During the whole procedure the cardiogram was followed on an oscilloscope and both this curve and a sound track with spoken comments were registered continuously by means of a specially constructed 4-channel tape recorder.

In all cases the initial shock was 100 watt seconds and if necessary new shocks of

TABLE II. Cardiac arrhythmias treated with synchronized DC-countershock. No. of shocks given for each treatment

No. of DC shocks	First treatment	Second treatment (5 days to 4 months later)	Third treatment (6 months later)
1	20	2	
2	9	2	
3	6	3	
4	16	3	1
5	4	—	
Total	55	10	1

increasing effect, 200, 300 and 400 watt sec, were applied.

All the eleven patients in whom reversion was not accomplished thus received 4 or eventually 5 shocks (table II).

After the counter-shock treatment all patients were given 500 mg quinidine daily until the first follow-up two months later. The anticoagulation treatment was stopped immediately after the defibrillation.

All patients alive and with sinus rhythm were seen at a follow-up investigation which included ECG 2—3 months after (31 patients) and again 6–15 months after the treatment (17 patients).

Results

It is shown in table I that conversion to sinus rhythm was obtained in 44 out of the 55 patients (80 %).

Relapse of the arrhythmia before discharge from the hospital occurred in six patients within the first few minutes, in 9 patients between one hour and five days after the treatment.

Among the patients whose arrythmia later on relapsed, nine received a second

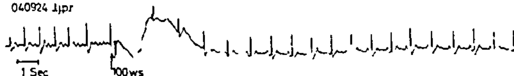


Fig 1 A case of atrial flutter in which the sinus rhythm follows immediately after the DC-shock

treatment and of them eight converted to sinus rhythm again In most of these cases the second conversion required a higher number of shocks, and the one patient who did not convert from the second treatment was the only one who received the maximum effect (400 watt sec) in the first treatment The patient who was treated three times on the first occasion needed only a DC-shock of 100 watt sec , on the second occasion 300 watt sec , but the third time the treatment failed even after 400 watt sec

At discharge from hospital 32 of the 55 patients treated (58 %) had sinus rhythm including one patient who died in the hospital from bronchial cancer, but without recurrence of the arrhythmia

At the first follow up 2—3 months later the ECG showed sinus rhythm in 19 (34 % of the patients treated) Two of these 19 patients died 9 and 10 months after the DC shock one in an attack of ventricular tachycardia and the other shortly after an acute coronary occlusion

The remaining 17 patients were examined again 6—15 months after the treatment and at that time 14 still had sinus rhythm Sinus rhythm persisted at this last follow up study in 27 % of the treated patients, including the patient who died in ventricular tachycardia but had sinus rhythm at the admittance to the hospital In the other three patients the arrhythmia recurred 3, 6 and 8 months after the treatment

It is seen from table I that the chance of a permanent result was considerably less when the arrhythmia had persisted for a long time Only 8 % of the patients with an arrhythmia of more than one year s duration kept their sinus rhythm A permanent sinus rhythm was a little more frequent in the younger patients 37 % of the patients below 50 years had sinus rhythm at the follow up, against only 23 % of patients over 50 years Furthermore it is shown that a good result was most frequent (45 %) in the group no other heart disease , in which 3/4 were men Both for the group valvular disease", which mostly consisted of women and for the group ' coronary disease ', it can be concluded that the permanent result of the treatment was poor, even if the immediate result was as good as the average Finally it is seen that the chances for a good long term result is definitely less in patients with a pronounced heart dilatation

Complications

Fig 1 shows a case in which the sinus rhythm followed immediately after the DC countershock In most cases, however, different types of cardiac arrhyth-

TABLE III Disturbances of cardiac function immediately following DC-shock

	First treatment	No reverted to sinus rhythm	Repeated treatment	No reverted to sinus rhythm
None	19	14	6	5
Extrasystoles supraventric	18	18	4	4
Extrasystoles ventric	11	8	—	
Atrial flutter	3	3		
A v block incomplete	3	3	—	
A v block complete	4	2	2	2
Asystole	3	2		
Total	55	44	10	9

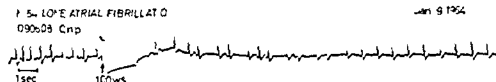


Fig 2 A case of atrial fibrillation in which supraventricular extrasystoles are seen in the first seconds after the DC-shock

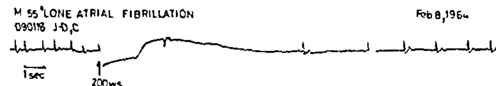


Fig 3 The DC-shock is followed by an asystole before the atrial fibrillation returns

mias and ectopic rhythms were seen (table III) Most common were extrasystoles during the first few seconds (fig 2) or up until a couple of hours after the shock In three patients the reversion from atrial fibrillation *to sinus rhythm* passed through a period of atrial flutter Three patients had sinus rhythm with a prolonged PQ interval lasting a few days and four patients first had a total a v block which only in two cases then reverted to sinus rhythm In a few cases an asystole of several seconds duration was seen immediately after the counter shock (fig 3) All these arrhythmias produced by the treatment passed spontaneously they were often of very short duration and did not give rise to extra therapeutic problems and most probably they had been without any influence on the circulation and final result It was not possible to show that the arrhythmias were especially common in those patients who had received more

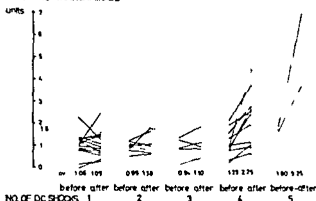


Fig. 4. The serum GO transaminase activity in 14 patients just before and 1—3 days after treatment with DC-shock.

and stronger shocks: it was rather a matter of the same type of arrhythmia returning when a new shock was given to the same patient.

No signs of embolism were seen in connection with the treatment.

Measurements of serum GO transaminase both before and three days after the treatment were available in 14 patients (fig. 4). Elevated values after the shock were seen in 13 patients, definitely more frequent and more pronounced in those who had received 4 or 5 shocks in the same treatment. Two of the patients with increased transaminase activity complained of oppression and soreness in the chest during the first week after the treatment. Electrocardiogram, sedimentation rate and leucocyte count, which were registered in all patients just before and after treatment, never showed abnormal values, and subjective symptoms of coronary disease or heart insufficiency never occurred. The elevation of transaminase level must be regarded as a consequence of the treatment and a warning that repeated DC countershocks might have undesirable influences on the myocardium.

Slight erythema of the skin, where the electrodes had been placed, was frequently seen, especially when repeated shocks had been given.

Discussion

Synchronized DC countershock has proven to be a highly effective and safe way of converting certain cardiac arrhythmias into sinus rhythm, and certainly in many cases will be the more attractive alternative to anti-arrhythmic drugs. The introduction of this treatment has therefore pushed to the fore a series of problems. In the following each of these questions will be considered, in relation to the by far most common type of arrhythmia, chronic atrial fibrillation with perpetual arrhythmia.

1. What *advantage*, if any, does the patient gain from conversion of his arrhythmia? Two abnormalities are implied in atrial fibrillation: atrial transport function is abolished, and ventricular rhythm has become irregular.

The haemodynamic significance of atrial systole has been much debated and has been made the subject of many investigations both in experimental animals and in clinical work (26 29) There is then, evidence that a properly timed and forceful atrial contraction does increase ventricular performance at rest, and probably more so when heart rate is elevated or ventricular filling impaired, as in stenosis of the cuspid valve or ventricular hypertrophy The differences so far measured, however, are small — e g a 5—15 % increase in cardiac output — and the influence under more extreme physiologic variations is not known (15, 26)

The significance of the other abnormality ventricular arrhythmia, is even more difficult to assess Irregular rhythm is generally thought to be a less economic way of performance because a systole following closely after the proceeding one must yield lower stroke volume for practically the same pressure work and oxygen consumption — but this can hardly be expressed in figures of general validity

The combined effect on the circulation of atrial fibrillation and perpetual arrhythmia has been investigated in several clinical papers where the same haemodynamic measurements could be made in groups of patients with and without atrial fibrillation but otherwise similar (9 27) or in the same patients before and after conversion to sinus rhythm (6 8 31) Results from such different studies of course are incomplete and not very comparable The available evidence however suggests that even if this type of arrhythmia may be of no importance to a normal heart and circulation at rest, it generally does interfere with the proper adjustment of cardiac frequency and cardiac output during exercise and especially under certain pathological conditions such as the aforementioned (3, 7) Obviously no simple or general answer exists to this question but an estimate must be made in every case, in which the alternatives to be compared are on one hand conversion to sinus rhythm which might be a very unstable state, and or the other hand persistence of the arrhythmia, but with all the aid that proper digitalization etc can offer This will be discussed futher under point 4

2 The *effectiveness* of synchronized DC-countershock in the meeting the acute need to convert atrial fibrillation into sinus rhythm has been the subject of several papers published during the last two years It appears from table IV that experiences have been rather uniform electrical defibrillation with this technique is able to convert about 80 to 90 per cent of cases of atrial fibrillation The small differences in the rate of immediate success have been attributed mainly to a different selection of patients, and to some extent to the influence of pre treatment with quinidine and to the technical details in the defibrillation itself

The alternative drug treatment generally applied in this contest is quinidine administered in the well known manner in daily increasing doses until defibrillation or symptoms of intoxication result or until an agreed maximum dose is reached. Table IV shows that the

TABLE IV Effect of quinidine and DC-defibrillation in conversion of chronic atrial fibrillation

		Immediate results			Late results		
	No of patients treated	Sinus rhythm in % of patients treated	Dose of quinidine (g/day) Max	Dose of quinidine (g/day) Average	Sinus rhythm in % of patients treated	Observation time	Maintenance dose of quinidine (g/day)
Quinidine treatment							
Viko et al (33)	75	68	4	—	34	Few days to 10 months	
Parkinson and Campbell (21)	44	68	3	1 5	52	Max 5 yrs av 2 yrs	
Yount et al (34)	155	76	—	2 5	[1]65	1—36 months	0 8
Sokolow and Ball (30)	177	73	6	2 2	[1]26	—	1 6
Friedberg and Sjostrom (5)	133	47	2	1 5	35	At dismissal	—
Mosbech and Thomsen (19)	87	45	2	—	—	—	—
Blondeau et al (2)	575	62	3	—	—	—	—
Rokseth and Storstein (24)	274	—	—	—	47	At dismissal	—
Sandoe et al (25)	100	58	3	—	[1]26	5—8 months	0
DC-defibrillation							
Lown (14)	161	93	—	—	—	—	—
Killip (11)	46	90	—	—	59	1—8 months	1 2
Oram and Davies (20)	100	84	—	—	40	1—11 months	0
Morris et al (18)	63	94	—	—	79	1 9 months	1 0
Miller and Winston Salem (17)	20	90	—	—	65	3 months	0 8 2 0
Cullhed et al (4)	27	70	—	—	—	—	0 8 1 6
Lemberg et al (13)	86	91	—	—	—	—	1 2
Hurst et al (10)	121	96		—	66	3 12 months	0 8
McDonald et al (16)	40	83		—	45	1—9 months	1 2
Rabbino et al (23)	35	88		—	32	2 months	1 2
Own material	48	79		—	25	6—15 months	0 5

[1] Calculated from follow up studies on some of the material

effectiveness of this treatment in accomplishing reversion to sinus rhythm was definitely less, the rate of immediate success varying between 45 and 75 % and clearly depending on the size of the maximum dose (2, 5, 19, 21, 25, 30, 33)

In these patients there is a well known tendency for the arrhythmia to relapse sooner or later after reversion

Table IV also shows that, in most of the series the number of sinus rhythms after a few months was reduced to half or less This rate of long term success undoubtedly bears little or no relation to the method by which defibrillation is acieved, DC countershock or quinidine, but is mainly dependent on two factors the type and degree of the cardiac

TABLE V Hazards of quinidine and DC-defibrillation in conversion of chronic atrial fibrillation

	No of patients	Cardiac arrest (%)		Embolic episodes (%)	
		Total	Those who died	Total	Those who died
Quinidine treatment					
Viko et al (33)	[1]484	1 2	1 2	1 9	0
Parkinson and Campbell (21)	[1]554	4 0	4 0	—	—
Askey (1)	[1]839	2 5	2 5		—
Thomson (32)	[1]611	3 3	2 1	2 3	0
Sokolow and Ball (30)	177	0 6	0 6	1 2	0
Friedberg and Sjostrom (5)	133	6 8	3 0	6 0	3 0
Mosbech and Thomsen (19)	87	1 2	1 2	1 2	1 2
Blondeau et al (2)	575	1 2	1 2	1 0	0
Rokseth and Storstein (24)	274	2 9	0	1 1	0
Sandoe et al (25)	100	2 0	0	0	0
DC-defibrillation					
Lown (14)	46	0	0	0	0
Killip (11)	161	0	0	0	0
Oram and Davies (20)	100	1 0	0	2 0	0
Morris et al (18)	63	1 6	0	3 2	0
Miller and Winston Salem (17)	20	0	0	0	0
Cullhed et al (4)	27	0	0	0	0
Lemberg et al (13)	86	0	0	0	0
Hurst et al (10)	121	0	0	1 6	0 8
McDonald et al (16)	40	0	0	0	0
Rabbino et al (23)	35	2 9	2 9	5 8	5 8
Own material	48	0	0	0	0

[1] Cases collected from literature

disorder, i e the proper selection of the patients as will be discussed below and the choise of maintenance treatment especially with quinidine Evidence exists that the chance of relapse was definitely reduced in the patients who were kept on a maintenance dose of not less than 1 6 g of quinidine per day

3 It is well established that certain *risks* are inherent in the process of converting atrial fibrillation back to sinus rhythm The serious hazards are mainly two embolic episodes and cardiac ectopic rhythms, especially ventricular tachycardia and fibrillation

In all known series of quinidine treatment, a few of which are shown in table V, cases of sudden death or transient syncope have been reported in between 0 5 to 4 % These serious complications occurred during drug treatment frequently just after conversion It has been emphasized several times (21, 30 32) that no embolus or other anatomical cause of sudden death could be found at

post mortem examination. Moreover, from animal experiments the cardiac effect of large quinidine doses is known to entail ventricular fibrillation (12), and in clinical work during recent years a ventricular tachycardia and fibrillation has been demonstrated several times during these syncopal attacks (25, 28). Accordingly, little doubt remains that most of the cases of sudden death or syncope occurring during quidinine treatment were not caused by an embolus or any neurogenic 'quinidine shock', but were due to serious disturbances in cardiac rhythm, especially ventricular fibrillation, as a direct effect of the large doses of quinidine. It should be stressed, however, that this is true only for the large and rapidly increasing doses of quinidine. When conversion is attempted with a moderate and constant maintenance dose such cases are hardly ever seen (30). During defibrillation with synchronized DC countershock provocation of ventricular fibrillation has been reported in only a few cases, and most of them have been stopped immediately by a new shock and the patient has survived. The DC treatment, therefore, seems to be superior to drug treatment, also in terms of a lower risk. It is a definite advantage, too, that the fatal cases do not occur unpredictably during the course of several days, as may be the case with quinidine treatment, but that the risk is confined to a few minutes where control is intensive, and the possibilities for immediate countermeasures are maximal.

Serious episodes of embolism in the brain or in the lungs have been reported during both forms of treatment. Present figures suggest that this complication, too, was probably more common during quinidine treatment than during electrical defibrillation. The difference may be partly due to a tendency, at least in the older series, to ascribe all cases of sudden death to embolic episodes. None of these series allow of conclusions as to the possible prophylactic value of anticoagulation treatment.

Minor side effects and inconveniences of the DC-defibrillation, such as transient but harmless cardiac arrhythmias, erythema of the skin, and a possible rise in serum GO transaminase, have been found in all series reported, as well as in the patients described here. These must be considered definitely minor in relation to the known nuisance of several days' treatment with large doses of quinidine.

4. *Indications* for treatment with synchronized DC countershock can thus be extended rather far, even to acutely ill patients. Therefore, the decision whether to apply this treatment in a case of atrial fibrillation would seem to depend on two questions only: in which patients can a conversion to sinus rhythm be expected to be of any benefit? and in which patients can a sinus rhythm be expected to last?

As mentioned above, the first question still needs further elucidation. It should be kept in mind, too, that a stable atrial fibrillation, well controlled by digitalis, might well be preferable to a state with repeated changes in rhythm.

The answer to the second question should emerge from follow up examina

tions as presented already in several series To summarize, it appears at present that the best chance to achieve a permanent sinus rhythm seems to exist in younger persons, in whom the arrhythmia has not been present for more than a year and who are not suffering from advanced cardiac disorders such as valvular lesions or coronary disease

Summary

Treatment with synchronized DC countershock was applied 66 times to 55 patients Fifty three patients suffered from a long standing atrial fibrillation or flutter 2 from a paroxysmal tachycardia Many were elderly patients and a few acutely ill

Conversion to sinus rhythm was obtained in 44 out of the 55 patients (80 %) in 53 out of the 66 treatments Sinus rhythm persisted at the time of dismissal in 32 (58 %), at follow up studies 2—3 months later in 19 out of surviving converted patients, and at the last examination 6—15 months after the treatment in 14 patients

The treatment was administered under full anaesthesia and with anticoagulation treatment, and no serious side reactions were observed A rise in serum GO transaminase level was seen in some cases after the defibrillation when repeated shocks were given Erythema of the skin and various arrhythmias and ectopic rhythms were frequent, but always transient and insignificant

Synchronized DC countershock seems to be to preferable as an alternative to quinidine treatment when conversion of an atrial fibrillation to sinus rhythm is desired The possible role of this procedure in clinical work is discussed

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"Juvenile" Xanthomatosis — a Recessive Inherited Disease?

By

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Essential hypercholesterolemia is a familial disorder characterized by an increase of cholesterol and phospholipids in serum, but with normal values for serum triglycerides Secondary, deposits of cholesterol can be seen in various organs, among others as cutaneous and tendinous xanthomas In general, xanthomic lesions manifest themselves initially at an older age but can also appear in childhood, being then often called "juvenile" xanthomatosis

In this article, three cases of "juvenile" xanthomatosis from two different families are reported Investigations of other family members as well, make a discussion of the inheritance of this disorder possible

Material and methods

The present material consists of three cases with juvenile xanthomatosis all of them diagnosed during hospitalization because of cutaneous deposits Investigations of 13 other family members were carried out in their homes For practical reasons only one nonfasting blood sample could be obtained from each Only cholesterol values definitely above the upper normal range are therefore accepted as hypercholesterolemic

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Case reports

Family I

Case 1 Girl born 1950 From the age of 12 months cutaneous and tendinous xanthomas developed as shown in fig 1 In 1962 xanthelasmas on both eyelids and incomplete arcus corneae were also found

Studies of her family revealed that neither her sister nor her twin brother showed any signs of cholesterol deposits in the skin or tendons Her mother and one maternal uncle showed small xanthomic nodules in the Achilles tendons Her father and the remaining 6 family members examined had no external xanthomic lesions The parents were first cousins Fig 2 gives the results of the lipoid studies in this family As seen from the figures all three children had elevated values for serum cholesterol The corresponding values found in the parents and their siblings will be discussed later

Family II

Case 2 Girl born 1949 At 2 years of age xanthomic nodules developed in both Achilles tendons Later xanthomatous plaques were found on the knees and elbows as shown in fig 3

Case 3 Boy born 1954 From the age of 5 years xanthomatous nodules were seen at the bases of 1 and 2 fingers increasing in size No other xanthomatous lesions were



Fig 1 Case 1 Xanthomic formation on the elbow

observed at the examination when 10 years old

Studies of the family revealed that two younger siblings and their parents had no xanthomatous nodules or plaques Both parents and at least three of the children however had definitely increased serum cholesterol values The parents were second cousins In fig 4 is given the results of the lipoid studies in this family

Results

The clinical picture of these three cases with extremely high values for serum cholesterol and with xanthomatous lesions in early childhood differs from what is usually seen in patients with essential hypercholesterolemia It may therefore be of considerable interest to compare the heterogenity in clinical manifestations with the underlying genetic mechanism

In each of the two families both parents had an increased level of serum cholesterol None of them had developed excessive xanthomatous lesions, but in one case small nodules on the Achilles tendons were found In both families the parents were consanguineous The occurrence of juvenile xanthomatosis in the siblings without manifestations of the disease in the parents, contradicts simple dominant inheritance On the other hand, manifestations of juvenile xanthomatosis among siblings of both sexes together with consanguinity between the parents strongly indicate an autosomal recessive inheritance

In family 1, definite hypercholesterolemia was also present in 3 of the parents siblings and one sibling had moderately

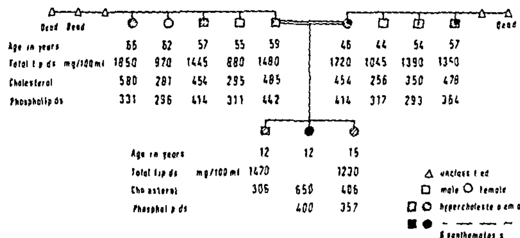


Fig 2 Family I Results of lipoid studies

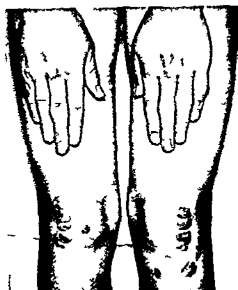


Fig 3 Case 2 Xanthomatic formation on hands and knees

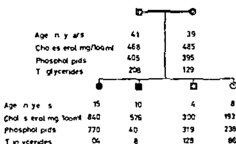


Fig 4 Family II Results of lipoid studies Symbols as in fig 2

elevated serum cholesterol Normal cholesterol concentrations were found in 3 other siblings Though small in numbers the data support the theory of simple dominant inheritance for this form of hypercholesterolemia without juvenile xanthomas

Similar observations are also made by other authors reporting cases of juvenile xanthomatosis In table I data from family investigations (4 5 6 7 10) on this subject are collected and compared with my own results

From such a review it is clearly seen that all children with juvenile xanthomatosis have extremely high levels for serum cholesterol They separate themselves from their siblings who have cholesterol values ranging from the normal levels (3) to values pathological for their age Conclusions about the incidence of juvenile xanthomatosis among siblings cannot be drawn from the figures partly because some of the unaffected children are still too young and partly because of the small number of siblings

In all families referred to in table I the parents had serum cholesterol above levels considered normal by the authors In none of the parents was excessive cutaneous or tendinous xanthomas present In addition to the here reported cases consanguinity between parents was reported in the case of Kornerup (5) and Khachadurian (4) who have investigated 10 families with juvenile xanthomatosis and found consanguinity in five while in four other families consanguinity could not be ruled out The incidence of intermarriage relationship is higher than usually found in other well known recessive inherited anomalies as albinism and Follings disease (phenylketonuria) and may indicate that juvenile xanthomatosis is a very rare disease

As formerly mentioned the familiar nature of the essential hypercholesterolemia is generally accepted but there is still controversy regarding the mode of inheritance It was previously stated by Muller (8) Kornerup (5) and others that the disease was transmitted as a

Table I The relationship between xanthomatosis and serum cholesterol level in some family investigations (4 5 6 7 10) and in the author's results

	Parents			Children (below 16 years old)			
				With xanthomatosis		Without xanthomatosis	
Author	Sex	Age	Serum choles (mg/100 ml)	Age	Serum-choles (mg/100 ml)	Age	Serum choles (mg/100 ml)
Author's own results							
Family I	♂	59	485	12	650	12	305
	♀	46	454			15	406
Family II	♂	41	468	15	840	14	300
	♀	39	485	10	576	8	193
Meilman (7)	♂	35	338	11	855	8	347
	♀	36	331			4	197
Kornerup (5)	♂	42	418	13	616	8	184
	♀	41	340				
McCleary (6)	♂	37	405	1½	801	9	277
	♀	29	390	7	860		
Wilkinson (10)	♂	49	466	11	700	14	254
	♀	49	288	3	550	12	394
						8	185
						1	381
Khachadurian (4)	♂	40	391	9	746	12	135
	♀	29	291			5	157
	♂	45	372	14	884		
	♀	35	327				

simple dominant trait This theory was supported by Piper and Orrild (9) who from their family investigations further concluded that the developement of xanthomas seemed to be closely correlated to the age of the patient and the serum cholesterol level In 1948 Wilkinson et al (10) pointed out that the disease was transmitted as an incomplete dominant trait This theory was later supported by Adlersberg et al (1) According to these investigators individuals with one single abnormal gene (heterozygotes) will develop hypercholesterolemia alone, while individuals with two abnormal genes (homozygotes) will develop both hypercholesterolemia and xanthomas This implies that the mode of inheritance for the disease will be simple dominant as regards the hypercholesterolemia, while hypercholesterolemia with xanthomas will be a recessively inherited disorder Our data very strongly supports this theory

Based on family investigations Khachadurian (4) concluded that xanthomas can also develop in the heterozygote individual In such cases the lesions will be small and develop late in life Assuming this view is correct "juvenile xanthomatosis can be recognized as a recessively inherited and the adult form on the other hand as an incomplete dominant disorder

A practical question in connection with these genetic considerations of the manifestation of "juvenile" xanthomatosis is whether or not it is possible to recognize the heterozygote individual with essential hypercholesterolemia from the population in general On fig 5 a comparison is given between the cholesterol values in serum from the 16 parents referred to in table I, and the Gaussian distribution curve for the serum cholesterol levels in what is called a "normal population (2) Although such a comparison between figures from different countries must be given with reservation it is clearly seen that all parents have cholesterol values that merge with values obtained from the "normal population Similar observations are also reported by Meilman et al (7) and Khachadurian (4) who emphasize that heterozygote individuals without xanthomatosis cannot with certainty be separated from the normal population

It appears however that in other recessive diseases in which heterozygote tests have been developed (galactosemia phenylketonuria) there is also some overlapping of the test values in the carriers and normal individuals

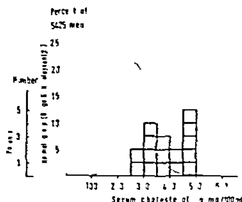


Fig 5 A comparison between the distribution curve for the cholesterol values in a "normal population (2) and the serum cholesterol found in parents to children with "juvenile xanthomatosis.

Summary

Excessive xanthomatosis together with hypercholesterolemia in three children from 2 families are reported Lipoid studies have been performed in a total of 16 family members In both families the parents were consanguinous and had increased levels of serum cholesterol but none of them had xanthomato is similar to that found in the children

Manifestation of juvenile xanthomatosis among siblings of both sexes together with consanguinity between parents strongly indicate an autosomal recessive inheritance for this disease A review of the literature concerning the subject supports this theory

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Takayasu's Arteritis and Ankylosing Spondylitis

Report of Four Cases

By

JOUKO A PALOHEIMO, HELJO JULKUNEN, PENTTI SILTANEN and AARO KAJANDER

Cardiac and aortic lesions are known to occur in patients with ankylosing spondylitis (6, 14, 19, 23, 25) Destructive lesions of the aortic wall, especially in the media, with dilatation of the aortic ring and valvular endocardium lead to the clinical picture of aortic valvular insufficiency (5, 25, 26) Cardiac lesions may be the cause of disturbances in atrioventricular and intraventricular conduction with partial or total a v block (4, 15 25) or bundle branch block (9) in the electrocardiogram

In some cases of ankylosing spondylitis an arteritis of small or medium sized arteries has been seen (7 27) but the so called Takayasu's arteritis (2, 13 21 22, 24) in association with ankylosing spondylitis has not been reported in the literature In a series of 26 cases of Takayasu's arteritis we have made an X ray examination of the lumbar spine in 23 cases in 4 of these the examination disclosed changes typical of ankylosing spondylitis These cases might be of interest in a discussion on Takayasu's obstructive arteriopathy as one of the autoimmune diseases

Material and methods

Case 1 The patient a 46 year-old farmer had tonsillitis several times before tonsillectomy in 1960 In connection with tonsillitis in 1960 he had arthritic symptoms and was treated with gold for 3 months with good results In the same year there again were swelling and pains in his right hand and pain and stiffness in the lumbar region and neck ESR was 47 mm/first hour Radial pulses were normal During the winter of 1963 he complained of loss of power and pricking sensations in *both* arms The radial pulses were weak In August 1964 all the symptoms became worse The arthritic pains in the right hand and the pain and stiffness in the neck and lumbar spine were aggravated The muscles of the arms were wasted and powerless

ESR was now 62 mm per first hour X ray showed erosions and sclerosis of the s i joints and one syndesmophyte in the spine

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TABLE I Laboratory findings of four cases of Takayasu's arteritis and ankylosing spondylitis

	Case 1	Case 2	Case 3		Case 4	
			1962	1964	1963	1964
ESR mm/hr	62 – 37	112 – 48	70	58	74	58
Hb/100 ml	11 3	10 2	9 5	11 1	11 2	11 2
Leucocytes/mm³	6 600	6 500	6 300	–	8 900	6 700
Eosinophils per cent	1	1 5	2 – 5	–	1 5	2 5
Thrombocytes/mm³ thous	245	–	704	–	385	250
Serol tests for syphilis	–	–	–	–	–	–
AST	100	160	80	250	125	220
ASTA	0 64	0 64	1 0	2 0	1 25	0 64
Waaler Rose titer	0	0	0	0	0	0
Latex	–	–	–		–	–
Thyroid antibodies	–	–		–		–
DNA *antibodies*	–	–	–	–	–	–
L E cells	+ once	–	–	–	–	–
Coombs direct	–	–				–
Coombs indirect	–	-				–
Total serum protein g/100 ml	6 9	6 9	7 1	6 6	7 4	7 6
— albumin per cent	51 3	52 9	47 6	56 2	58 6	52 8
— alfa 1 globulins per cent	6	6	6	3	4	7
— alfa 2 globulins per cent	9	11	11	9	10	10
— beta globulins per cent	12	12	16	13	12	11
— gammaglobulins per cent	22	18	20	18	16	20
Fibrinogen mg/100 ml	520	480	645		580	555
Coagulation factors¹	Normal	Normal	Normal	Normal	Normal	Normal
Serum cholesterol mg/100 ml	152	315	231		189	191
PBI gamma g/100 ml		5 3	5 5		5 7	6 4
Immunoelectrophoresis	Normal	Immuno-glob slightly incr	Immuno glob incr		Immuno glob slightly incr	

¹ Coagulation factors studied Quick P and P factor V and VII recalcification time prothrombin consumption test thromboplastin generation test

The patient had lost 10 kg of weight during the previous year Vision had become worse and he suffered from headaches The radial pulses were very weak sometimes not palpable Gold and phenylbutazone effected improvement ESR dropped to 43 mm per first hour The diagnosis of ankylosing spondylitis with obstructive arteritis was made and the patient was sent for further examination

The physical examination revealed a slightly underweight man with a rigid spine The muscles of the upper extremities were wasted The thyroid gland was slightly enlarged and of firm consistency No heart murmurs were audible On palpation of the arteries the left temporal artery was weaker than the left the subclavian, axillary and radial arteries were not palpable but the left radial artery was sometime later felt

Fig. 1. Arteriographic findings in four cases of Takayasu's arteritis and ankylosing spondylitis in the same patient. Auscultatory findings in the areas not visualized in aortography are presented with horizontal lines.



Fig. 2. Arteriography in case 1. The aorta and its main branches are seen from the ascending aorta to the femoral arteries.

very weakly The consistency of the arteries was *firmer than normal, especially that of* both carotid arteries and their bifurcations On auscultation a systolic murmur was heard on both sides of the neck and in the epigastrium between the ensiform process and the umbilicus There also was a slight murmur over both femoral arteries Blood pressure could be measured in the legs only and was 240/140 No calcification was seen in X ray in these arteries The lungs were normal The heart volume was 510 cm^3/m^2 and the aorta was slightly dilated ECG showed a normal P Q time (0 19 s) and a partial right bundle branch block Retinal arteries were slightly narrowed In ophthalmodynamometry the pressure in the right eye was 90 mm Hg and that of the left 70 mm Hg, both lower than normal values X ray of s i joints and spine showed the typical changes of ankylosing spondylitis The results of the laboratory studies are seen in table I Aortography was done It was seen (figs 1 and 2 that both subclavian arteries were obstructed, the left carotid artery was slightly stenotic and the left vertebral artery stenosed The right *vertebral artery was narrow There were* three renal arteries to the left kidney and the root of the largest one was stenosed In the right renal artery there was slight stenosis and a poststenotic dilatation The aorta itself was normal except for a slight dilatation of the ascending part and the iliac arteries showed no gross changes Arterial biopsies were not taken

The patient was recommended for surgical treatment An endarterectomy of the right subclavian artery was made The vessel was *inflamed and the surrounding periarteri*al tissue fibrosed and stiff The vessel was opened and endarterectomy was performed The white mass removed from the vessel in *endarterectomy was of thrombus-like con*sistency Microscopical examination showed an organizing or organized thrombus covered with thickened arterial intima There was some inflammatory reaction in the thrombus and also some inflammation in the intima The elastic layer of the intima was in a good condition except for some slight degenerative changes Other layers of the arterial wall were not seen in *the specimen*

After the operation the pulsations of the left radial artery remained as before but those of the right radial artery were distinctly more powerful The patient had subjective help from the operation and there were no signs of activation of the arterial process He is under continuous anticoagulant therapy

Case 2 This patient a 24 year old married woman for 3 years had aching pains under the chin and in the neck lasting from 1/2 to 1 hour 3 to 5 times a day The attacks were brought about especially by the drinking of cold Pilsener Menstruation was normal and she had two children born in 1960 and 1964 by normal delivery In the summer of 1963 she was admitted to a hospital for general malaise and a high ESR of 94 mm per first hour A wisdom tooth was extracted and she was given antibiotic therapy The ESR declined to 48 mm per first hour After a normal pregnancy and delivery she again became worse and the ESR rose from 48 mm per first hour in November 1964 to 112 in January 1965 The general malaise and pains continued Her left arm and hand were sometimes colder than normal and she had dyspnea when walking upstairs

Physical examination revealed a slightly overweight young woman of normal build The muscles were normal The right lobe of the thyroid gland was slightly increased in size but of normal consistency In the heart there was a systolic murmur in the aortic region continuing to the neck On the palpation of arteries the pulsations were normal *except for a thrill at the right carotid* bifurcation In the auscultation a 3/6 grade systolic murmur was heard over the right supraclavicular area and a thrill was felt at the right carotid bifurcation A weak systolic murmur was audible over the abdominal aorta at the height of the umbilicus Blood pressure from both arm was 125/75 mm Hg X ray of lungs and heart revealed a normal situation ECG was normal The retinae showed normal vessels X ray of s i joints revealed marked calcification with a

narrowed joint slit typical of ankylosing spondylitis and periostitis near two costo-transversal joints The laboratory findings are presented in table I Biopsy of the temporal artery gave normal result Aortography (fig 1) showed that the right subclavian artery was narrowed at the base with a poststenotic dilatation distal to the stenosis Other vessels including those in the abdominal area showed no changes The bifurcations of the carotid arteries were not well seen in the arteriograms

This patient was given corticosteroid therapy with a good general result the impression after some weeks therapy being that the murmurs also were disappearing Anticoagulants are in use in this case too

Case 3 The patient a 25-year-old woman a telephone operator, had a brother of 17 with serious rheumatoid arthritis of juvenile type This woman had had attacks of tonsillitis before tonsillectomy in 1954 In that year she was examined at a tuberculosis sanatorium for back pain in the interscapular region general malaise anemia and high ESR Radial pulses were normal The pain continued without any clear etiology and a high ESR between 20 and 120 mm per hour was repeatedly measured In 1958 she was in the same sanatorium because of a high ESR and an infiltration in the upper lobe of the right lung The changes in the lung and the ESR were unchanged after six months antituberculosis treatment No bacilli were detected The radial arteries were not palpable She has been anemic since then Menarche and menstruation have been normal and she has had two children by normal delivery in 1961 and 1962 Two weeks after the last delivery there was an onset of rheumatic fever with migrating swelling and pain in the left knee right acromioclavicular joint and the elbows High fever up to 39 C ESR 78 mm/1 hour hypochromic anemia and leukocytosis up to 11 000 were seen The LE-cell phenomenon was negative and the Waaler Rose titer was 32 Radial arteries were not palpable With cortisone and antibiotic therapy the acute symptoms disappeared ESR dropped to 27 mm per hour, but a slight general malaise, slight dizziness in the morning irritability and recurrent arthritic symptoms in the knee joints continued

The physical examination showed a young woman of normal build The thyroid gland was normal in size and consistency Koilonychia was seen with anemic mucous membranes The muscles were normal The left ventricle of the heart was hypertrophic and a systolic murmur of grade 2/6 was heard over the aortic region On palpation of the carotid arteries a thrill was palpable The subclavian axillary and radial arteries were not palpable Other arteries pulsated normally but all were firmer than normal arteries On auscultation a systolic murmur was heard in the neck being very loud on the left side A slight systolic murmur was also heard over the abdominal aorta and a moderate one over the femoral arteries and in the back over the left kidney Blood pressure could not be measured from the arms but was 200/100 in the leg No calcification was seen over the arteries in X ray In the lungs there were fibrotic changes most possibly after tuberculosis in the anterior segment of the upper lobe of the right lung and a calcified paratracheal gland were seen Heart size was 505 ml/m² with a hypertrophied left ventricle In the ECG the P Q time was lengthened to 0 24 sec and QRS was slightly notched The eyegrounds were normal and the ophthalmodynamometry values were right 90 60 and left 100 60 both being normal X ray of s i joints showed sclerosis with uneven joint slits and narrowing and sclerosis were seen in the intervertebral joints of the lumbar spine The laboratory results are seen in table I

In aortography (fig 1) neither subclavian artery was visualized and the left vertebral artery also was obstructed In the left carotid artery there was stenosis with a long post stenotic dilatation The right carotid artery was slightly narrowed Arterial biopsies were not taken Muscle and skin biopsy specimens were normal

The patient was given cortisone therapy and continuous anticoagulant therapy with good response e g ESR fell to 28 and the fever disappeared. The disease has been active, as is seen from the laboratory tests made two years later The subjective symptoms are the same as before.

Case 4 This patient, a 24 year-old female university student had an attack of tonsillitis when about 12 years old As a schoolgirl she occasionally had pains in the joints of the knees and feet. Some years afterwards her left arm ached Aching pains in the hips had been examined several times Two years before the first examination in 1963 she had anaemia, general malaise and a high ESR the cause of the symptoms was thought to be pyelonephritis The left radial pulse was not palpable ESR was 40 and did not fall significantly although the general symptoms disappeared In 1963 the tiredness returned and the ESR was now 74 mm per hour Pyuria without bacteriuria was detected The left radial artery was not felt and the arm tired during working She was sent to the hospital for further examinations

The physical examination revealed a woman of normal build The muscles were normal and the thyroid gland was of normal size and consistency No murmurs were audible in the heart. On palpation the left subclavian, axillary and radial arteries were not felt but sometimes a weak radial arterial pulsation was palpable A systolic thrill was felt over the right carotid artery and in the supraclavicular region a pulsation was seen. A loud systolic murmur was heard over both carotid and subclavian arteries a moderate systolic murmur over the abdominal aorta and a slight one over the femoral arteries BP was 135 60 in the right arm, 80/70 in the left, and 135/70 in the legs X-rays showed normal lungs and a heart size of 375 ml/m² No calcification was seen in the arteries X-rays of the s-i joints and the lumbar spine showed sclerosis and uneven joint slits The results of laboratory studies are shown in table I

In aortography (fig 1) it was seen that the right subclavian artery was stenosed, with poststenotic dilatation both common carotid arteries were narrow, the left subclavian artery was very narrow 2 cm from its root and there were collaterals to the left arm. The root of the left vertebral artery was narrow and the artery dilated beyond the stenosis Arterial biopsies were not taken

Studies of the etiology of the pyuria gave no positive results The patient has received intermittent corti-costeroid therapy and continuous anticoagulant therapy The disease is still active as is seen from the laboratory results taken 2 weeks after a temporary discontinuation of corticosteroid

Discussion

The four patients presented were diagnosed as Takayasu's arteritis and ankylosing spondylitis One of them (case 1) had had active spondylitis 3 years before signs of obstructive arteritis appeared as "pulseless disease' The other three, all woman, had had systemic symptoms general malaise, a high ESR for 2 to 8 years and signs of arterial obstructions for some months to 7 years, as well as changes typical of ankylosing spondylitis at the time the examinations for arterial disease were made but without subjective symptoms for the s i joints in two of them

The etiology of Takayasu s arteriopathy is not known but the mechanism that seems nowadays most acceptable is the autoimmune process (e g 11, 13, 18, 24) The clinical picture of general malaise high ESR fever and symptoms of arthritis and rheumatic fever, is of the same type as the symptomatology of the autoimmune diseases LED, rheumatoid arthritis and ankylosing spondylitis

This clinical picture was seen in our patients, also The results of laboratory studies which are seen in table I, showed only some "positive" results ESR was high in all of the four patients and the LE cell phenomenon was positive transiently in case 1 The tests for thyroid antibodies and antibodies against DNA, the latex and Waaler-Rose tests and the Mantoux skin tests were negative The serum fibrinogen level was high as is common in collagen diseases, and this together with thrombocytosis in cases 3 and 4, may have been a factor increasing the intra arterial thrombosis of the inflamed artery Antibodies against human heart tissue could not be found (12) which is in accordance with the studies on aortic antibodies of Hirsch et al (11) in five cases of Takayasu's arteriopathy Possibly the antibodies could not be demonstrated with the techniques used

The systemic nature of the arterial changes in Takayasu s arteritis was seen in our patients In addition to changes in the aortic wall (especially in case 3) there were arterial stenoses in the branches of the aortic arch and in the renal arteries and, based on auscultation, in the iliac and femoral arteries also This multiplicity of changes has recently received much attention and it has been considered one point speaking for an autoimmune systemic disease, especially since changes have also been detected in the pulmonary arteries (24) The possibility of pulmonary arteritis cannot be excluded in case 3, who had had atypical tuberculosis of the right lung without bacteriological or therapeutic evidence of specific etiology

The histological changes seen in Takayasu's arteritis are those of panarteritis, with greatest changes in the media Degeneration and fragmentation of elastic fibers with infiltration of plasma cells and lymphocytes and sometimes of giant cells have been seen This feature is the same as that seen in the lesions of autoimmune diseases (17) Similar changes are seen also in the aorta in cases of ankylosing spondylitis and rheumatoid arthritis with aortitis (26, 27) In case 2 the temporal artery biopsy was negative and in case 3 the muscle and skin biopsy specimens were negative A histological study of the thrombus like mass excised at the operation of patient case 1 showed thrombotic changes, thickened intima and a slight inflammatory reaction

Our patients showed an excellent response to corticosteroid therapy This reactivity seems to be more or less specific to all autoimmune diseases (17)

The prescence of two such rare diseases as ankylosing spondylitis and Takayasu s arteritis cannot be considered to be a coincidence and obviously they should be regarded as two variants of one disease Takayasu s arteritis has been encountered also in association with LED (16, 20), with rheumatoid arthritis (8, 22) and with polymyalgia rheumatica (1 10) all of which are diseases with an assumedly autoimmune base The name and the nature of autoimmune disease may be dependent on the number of forbidden clones and thus variation and overlapping of the clinical features and laboratory findings are therefore possible

Summary

Four cases of Takayasu s arteritis in patients with ankylosing spondylitis are reported These patients were part of a series of 26 cases of Takayasu s arteritis, in 23 of which X rays were taken of the lumbal spine

One of the patients (male, aged 46) had had active spondylitis three years prior to signs of arterial disease The other three patients were woman (aged 24, 25, 24) with slight roentgenographic changes in the s i joint and spine and severe arterial changes in two of them and slight changes in one Arterial changes were seen in branches of the aortic arch, ascending and descending aorta, abdominal aorta, renal arteries, and iliac and femoral arteries ESR was elevated in all of the patients, the fibrinogen level was higher than normal and in two patients thrombocytosis was present The LE cell phenomenon was *positive in one* The *following* gave negative results AST, ASTA, Waaler-Rose, latex fixation, DNA anti body, antithyroid microsome and anti-*thyroglobulin antibody tests In electro* phoresis and immunoelectrophoresis the immunoglobulins were slightly elevated Antibodies against human heart antigens were not detected in the serum of these patients

The autoimmune character of the Takayasu's arteritis is discussed The concomitant presence of two such rare diseases as Takayasu s arteritis and ankylosing spondylitis in the same patients cannot be thought to be only a coincidence, especially since Takayasu s arteritis has been earlier reported in patients with autoimmune disease

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Hemodynamic Studies on the Hypotensive Effect of Acetabuton

A New Potent Sympatholytic Agent

By

RUNE SANNERSTEDT

Acetabuton (= Ac) has in animal experiments been shown to be a potent adrenolytic and sympatholytic agent with an activity of about 20 times that of dibenamine (2) Besides a marked hypotensive action Ac given to normotensive anesthetized dogs causes tachycardia and increased cardiac output, in this respect being different from sympathetic blocking agents like guanethidine and bretylium (8 9)

Ac has been synthesized at the Research Laboratory Dr C Janssen Chemically the compound belongs to the series of basic butyrophenones also including i a haloperidol

The present paper reports on the hemodynamic effects at rest and during exercise in the upright position of Ac given orally for 6—8 days to hypertensive patients

Material

Ten in patients with arterial hypertension of different causes and stages were studied (table I) Six of them were not previously treated with hypotensive agents The remaining four (pat O E H G S K and M P) had for a short period received treatment for their hypertension These drugs were withdrawn before the study started and were judged not to influence the trial

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Method

After 2—8 days of hospital rest the pretreatment study was performed in the morning with the patients in the fasting state Under local anesthesia and by the percutaneous route catheters were introduced into the brachial artery and the right atrium or a central vein

After a resting period of about 30 min an initial set of values was obtained with the subjects sitting comfortably in a chair The intravascular pressures were recorded on an Elema Electrocardiograph EM 130 *the mean arterial pressure being derived* from electrically integrated curves The cardiac output was determined using a dye dilution technique with bromsulphalein as the indicator according to Wassén (11) and Forsberg (3) The heart rate during the dye procedure was taken from a simultaneously recorded ECG The oxygen consumption was measured by sampling of expired air in

TABLE I Clinical data on 10 hypertensive patients investigated hemodynamically before and during treatment with acetabuton

Pat	Age	Keith Wagener group	Diagnosis	BP on admission (mm Hg)		Daily dose of acetabuton	
				Lying	Standing	mg	days
♂							
E E	46	II	EH	175/125	—	20	7
O E	64	II	EH	240/150	250/165	20	7
H G	57	I	EH	180/105	180/120	15	7
G H	52	II	EH	240/150	230/155	25	8
T J	45	0	EH	190/100	190/110	20	7
S K	52	II	Renal artery stenosis	205/105	205/110	20	8
F N	58	II	Renal artery stenosis	245/115	235/130	20	7
M P	39	0	Labile hypertension	175/95	—	15	6
♀							
V E	36	I	Post toxemic hypertension	220/130	205/135	15	7
K J	52	I	EH	190/95	205/130	15	7

Abbrev EH – essential hypertension

a Douglas bag with subsequent gas analyses in duplicate

Two or three exercise periods on different work loads with a resting interval of 10—15 min between each were then performed with the patients in the sitting position on the bicycle ergometer described by Holmgren and Mattson (5) The results presented are derived from the second period where the load setting was 600 kpm/min for the males except for pat G H and F N who exercised 400 and 300 kpm/min respectively The second work load setting for the females was 400 kpm/min Sampling of expired air was started after 5—6 min of exercise The dye injection for cardiac output determination preceded by intravascular pressure recordings was made after 9 min except in pat K J where it occurred after 6 min In six patients the intraarterial pressure was recorded two minutes after terminating the exercise while still sitting on the bicycle

Ac was then administered orally in divided doses for 6—8 days The daily dose was adjusted according to the hypotensive answer and amounted to 15—25 mg No other hypotensive agents were given Side effects of any importance were not seen

Thereafter the hemodynamic study as described above was repeated under the same conditions and with the same techniques The work load settings were the same as in the first study For technical reasons the exercise study could not be performed in pat T J

In analysing the differences between the two studies Student's t test was used

Results

At rest The findings are presented in table II and a representative case is shown in fig 1 The heart rate during treatment with Ac was unchanged The systolic, diastolic and mean brachial artery pressures were significantly lower

(P < 0.01) at the second study, the mean differences being —30/—15,—18 mm Hg.

As there was at the same time a small but probably significant (P < 0.05) increase in the cardiac output, the calculated total peripheral resistance decreased markedly with an average of —5.1 arbitrary units (P < 0.01).

The stroke volume was almost unchanged as was also the oxygen consumption. The hematocrit showed a highly significant (P < 0.001) decrease from an average of 41 % to 36 %.

During exercise. The findings are presented in table III and a representative case is shown in fig. 1. The increase in heart rate during the exercise test was the same in both studies. The recorded brachial artery pressures were significantly lower after the administration of Ac. On an average the systolic pressure decreased 44 mm Hg (P < 0.01), the diastolic 26 mm Hg (P < 0.001) and the mean arterial pressure 34 mm Hg (P < 0.001).

During exercise there was also an increase in the cardiac output at the second study (+ 1.0 l/min), the difference was, however, not significant. Accordingly there was a fall in the calculated peripheral resistance, the difference being highly significant (P < 0.001).

The stroke volume and oxygen consumption did not show any consistent changes. As at rest the hematocrit was significantly lower at the second study.

No patient showed any tendency to develop hypotension when exercising. The blood pressure fall immediately

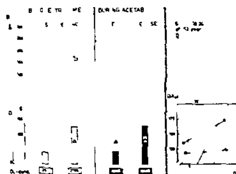


Fig. 1. Results from a male patient with renal artery stenosis treated orally with 5 mg q. i. d. for eight days. (Abbrev. see table II.)

after exercise was watched in seven subjects. One patient (S.K.) had a rapid pressure drop and the recording had to be stopped after a few seconds because of impending fainting. In the other six patients the difference in mean arterial pressure recorded two minutes after terminating the exercise was of the same magnitude as the difference found during the exercise periods.

Discussion

Treatment with acetabuton (Ac) — a new adrenolytic and sympatholytic agent — afforded in this study a substantial decrease of the arterial pressure with maintained or slightly increased cardiac output.

The differences found between the first study before treatment and the second during treatment with Ac might have been due to the effect of a prolonged hospital stay. Previous studies at our laboratory have, however, shown almost identical results when hemodynamic studies were repeated on patients while staying in the hospital (10).

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T J	45	0	EH	190/100	190/110	20	7
S K	52	II	Renal artery stenosis	205/105	205/110	20	8
F N	58	II	Renal artery stenosis	245/115	235/130	20	7
M P	39	0	Labile hypertension	175/95	–	15	6
♀							
Y E	36	I	Post toxemic hypertension	220/130	205/135	15	7
K J	52	I	EH	190/95	205/130	15	7

Abbrev EH = essential hypertension

a Douglas bag with subsequent gas analyses in duplicate

Two or three exercise periods on different work loads with a resting interval of 10—15 min between each were then performed with the patients in the sitting position on the bicycle ergometer described by Holmgren and Mattson (5) The results presented are derived from the second period, where the load setting was 600 kpm/min for the males except for pat G H and F N who exercised 400 and 300 kpm/min respectively The second work load setting for the females was 400 kpm/min Sampling of expired air was started after 5—6 min of exercise The dye injection for cardiac output determination, preceded by intravascular pressure recordings was made after 9 min except in pat K J where it occurred after 6 min In six patients the intraarterial pressure was recorded two minutes after terminating the exercise while still sitting on the bicycle

Ac was then administered orally in divided doses for 6—8 days The daily dose was adjusted according to the hypotensive answer and amounted to 15—25 mg No other hypotensive agents were given Side effects of any importance were not seen

Thereafter the hemodynamic study as described above was repeated under the same conditions and with the same techniques The work load settings were the same as in the first study For technical reasons the exercise study could not be performed in pat T J

In analysing the differences between the two studies Student s t test was used

Results

At rest The findings are presented in table II and a representative case is shown in fig 1 The heart rate during treatment with Ac was unchanged The systolic, diastolic and mean brachial artery pressures were significantly lower

CO		TPR		SV		O_2 cons		Hct	
B	Ac	B	Ac	B	Ac	B	Ac	B	Ac
5 2	6 1	26 4	18 2	91	87	260	305	41	37
5 1	4 9	29 8	26 6	74	70	—	—	42	36
4 9	4 7	22 3	20 4	70	73	298	252	42	38
—	—	—	—	—	—	317	311	46	36
5 3	6 0	21 9	18 5	96	97	326	219	44	42
3 7	4 4	36 5	26 8	69	75	203	234	40	35
6 3	6 3	24 8	21 3	98	94	275	279	40	34
8 0	8 5	15 3	12 9	68	77	284	286	45	38
4 9	6 8	32 0	21 6	55	70	236	257	37	32
6 1	7 3	19 2	15 8	90	94	134	211	35	34
9		9		9		9		10	
5 5	6 1	25 3	20 2	79	82	259	261	41	36
+0 6		−5 1		+3		+2		−5	
<0 05		<0 01		>0 1		>0 1		<0 001	

TPR total peripheral resistance (arbitr units)
SV = stroke volume (ml/beat)
O_2 cons = oxygen consumption (ml/min)
Hct = hematocrit (%)

ed plasma volume in conformity with drugs like pentolinium and guanethidine (6) Seven out of ten patients increased in weight during treatment with Ac, averaging + 0 4 kg Clinical signs of fluid retention were however, not seen in any case

Ac is a pharmacologically interesting drug and has in this short term study been shown to be a potent and well tolerated hypotensive agent in arterial hypertension with a probably peripheral site of action Studies on its effect in prolonged treatment have however shown the development of resistance (4), and occurrence of troublesome side effects (7) The present results indicate nevertheless that drugs of similar chemical composition should be tried as hypotensive agents

Summary

1 The hemodynamic effects of acetabuton — a new potent adrenolytic and sympatholytic agent — have been studied in ten hypertensive in patients

TABLE III Hemodynamic findings during exercise before (= B) and during treatment with aceta-

	HR		BA_S		BA_D		BA_M		CO	
Pat	B	Ac	B	Ac	B	Ac	B	Ac	B	Ac
♂										
E E.	160	164	235	193	127	97	165	124	14 5	16 5
O E	152	139	295	229	119	99	165	141	12 3	14 4
H G	138	136	213	170	97	77	136	111	13 8	15 0
G H	120	136	270	200	137	87	188	131	14 9	13 9
S K	163	155	291	205	115	83	173	123	12 5	12 8
F N	123	128	286	240	140	100	201	150	11 9	12 3
M P	185	184	190	187	90	81	126	112	12 9	17 0
♀										
V E	169	176	250	210	126	94	174	138	12 0	13 4
K J	156	165	222	222	96	94	151	140	13 3	11 6
n	9		9		9		9		9	
M	152	154	250	206	116	90	164	130	13 1	14 1
diff	+2		−44		−26		−34		+1 0	
P	>0 1		<0 01		<0 001		<0 001		>0 1	

Abbrev see table II

2 The intraarterial blood pressures and the cardiac output at rest and during exercise in the upright position were determined before and after oral administration of 15—25 mg daily for 6—8 days

3 The arterial blood pressure was significantly lower both at rest and during exercise at the 2nd study, the differences in mean arterial pressures being — 18 and — 34 mm Hg respectively Concomitantly the cardiac output was unchanged or slightly increased giving significant reductions in calculated peripheral resistance There were no changes in the heart rate or stroke volume The hematocrit was significantly lowered, probably due to plasma volume expansion

4 The results indicate a peripheral site for the hypotensive action

Acknowledgement

Acetabuton was provided by AB Leo Halsingborg Sweden

References

1 DOLLERY C T EMSLIE SMITH D & MILNE M D Clinical and pharmacological studies with guanethidine in the treatment of hypertension. Lancet 2 381 1960

2 EXLEY K A Preliminary communication

buton (=Ac)

TPR		SV		O_2-cons		Hct		BA_M 2 after exercise	
B	Ac	B	Ac	B	Ac	B	Ac	B	Ac
11 4	7 5	91	101	1 533	1 701	45	41	129	86
13 4	9 8	81	104	—	—	46	39	—	—
9 9	7 4	100	110	2 128	2 101	46	42	115	93
12 6	9 4	124	102	1 606	1 460	49	40	162	112
13 8	9 6	77	83	2 196	2 064	44	38	—	—
16 9	12 2	97	96	1 273	1 194	42	36	170	110
9 8	6 6	70	92	2 003	1 730	46	40	99	87
14 5	10 3	71	76	—	—	42	33	—	—
11 4	12 1	85	70	—	—	38	36	91	75
9		9		6		9		6	
12 6	9 4	89	93	1 790	1 708	44	38	128	94
−3 2		+4		−82		−6		−34	
<0 001		>0 1		>0 1		<0 001		<0 01	

3 FORSBERG S Å Pulmonary blood volume in man A study using the double indicator technique in patients with cardiovascular disease Acta med scand Suppl 410 1964

4 GARGANO N & BARTORELLI C Preliminary communication

5 HOLMGREN A & MATTSON K H A new ergometer with constant load at varying pedalling rate Scand J clin Lab Invest *6* 137 1954

6 RØNSON JESSEN V Blood volume during treatment of hypertension with guanethidine Acta med scand *174* 307 1963

7 SABROE J Personal communication

8 SCHAPER W K A & JAGENEAU A H M Der Einfluss blutdrucksenkender Substanzen auf Elektrokardiogramm Blutdruck und Herzfrequenz nicht narkotisierter Hunde Arzneimittel Forsch *11* 1102 1961

9 SCHAPER W K A JAGENEAU A H M & XHONNEUX R Hemodynamic respiratory and renal responses to Acetabuton (R 3248) a new potent hypotensive agent in intact anesthetized dogs Arzneimittel Forsch *12* 1015 1962

10 SCHRODER G Reproducibility of hemodynamic studies repeated at a few day s interval Scand J clin Lab Invest *16* 559 1964

11 WASSÉN A The use of bromsulphalein for determination of the cardiac output Scand J clin Lab Invest *8* 189 1956

TABLE III Hemodynamic findings during exercise before (=B) and during treatment with aceta-

	HR		BA_S		BA_D		BA_M		CO	
Pat	B	Ac	B	Ac	B	Ac	B	Ac	B	Ac
♂										
E.E.	160	164	235	193	127	97	165	124	14.5	16.5
O.E.	152	139	295	229	119	99	165	141	12.3	14.4
H.G.	138	136	213	170	97	77	136	111	13.8	15.0
G.H.	120	136	270	200	137	87	188	131	14.9	13.9
S.K.	163	155	291	205	115	83	173	123	12.5	12.8
F.N.	123	128	286	240	140	100	201	150	11.9	12.3
M.P.	185	184	190	187	90	81	126	112	12.9	17.0
♀										
V.E.	169	176	250	210	126	94	174	138	12.0	13.4
K.J.	156	165	222	222	96	94	151	140	13.3	11.6
n	9		9		9		9		9	
M	152	154	250	206	116	90	164	130	13.1	14.1
diff	+2		−44		−26		−34		−1.0	
P	>0.1		<0.01		<0.001		<0.001		>0.1	

Abbrev. see table II

2. The intraarterial blood pressures and the cardiac output at rest and during exercise in the upright position were determined before and after oral administration of 15—25 mg daily for 6—8 days.

3. The arterial blood pressure was significantly lower both at rest and during exercise at the 2nd study, the differences in mean arterial pressures being −18 and −34 mm Hg respectively. Concomitantly the cardiac output was unchanged or slightly increased giving significant reductions in calculated peripheral resistance. There were no changes in the heart rate or stroke volume. The hematocrit was significantly lowered probably due to plasma volume expansion.

4. The results indicate a peripheral site for the hypotensive action.

Acknowledgement

Acetabuton was provided by AB Leo, Halsingborg, Sweden.

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2. EXLEY, K. A.: Preliminary communication

buton (=Ac)

TPR		SV		O_2-cons		Hct		BA_{AI} 2 after exercise	
B	Ac	B	Ac	B	Ac	B	Ac	B	Ac
11 4	7 5	91	101	1 533	1 701	45	41	129	86
13 4	9 8	81	104	—	—	46	39	—	—
9 9	7 4	100	110	2 128	2 101	46	42	115	93
12 6	9 4	124	102	1 606	1 460	49	40	162	112
13 8	9 6	77	83	2 196	2 064	44	38	—	—
16 9	12 2	97	96	1 273	1 194	42	36	170	110
9 8	6 6	70	92	2 003	1 730	46	40	99	87
14 5	10 3	71	76	—	—	42	33	—	—
11 4	12 1	85	70	—	—	38	36	91	75
9		9		6		9		6	
12 6	9 4	89	93	1 790	1 708	44	38	128	94
−3 2		+4		−82		−6		−34	
<0 001		>0 1		>0 1		<0 001		<0 01	

3 Forsberg S Å Pulmonary blood volume in man A study using the double indicator technique in patients with cardiovascular disease Acta med scand Suppl 410 1964

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From First Medical Service Sahlgren s Hospital University of Goteborg Sweden

Effects of Ethacrynic Acid on Renal Function in Man

By

GORAN BOJS and OVE LUNDVALL

Ethacrynic acid (2,3 dichloro 4 (2 methylenebutyryl) phenoxyacetic acid) is a new saluretic agent, structurally unrelated to benzothiadiazine diuretics or other previous diuretics (11)

Clinical trials in patients with fluid retention have shown that the drug when administered by mouth, is a potent saluretic agent often effective in patients refractory to other diuretics (3 a 6, 7, 8) According to Maher et al the drug in high doses is also effective at low filtration rates

As little has been published about the acute effects of ethacrynic acid given parenterally in man and as the drug given intravenously has been shown to be valuable in the treatment of pulmonary edema (5 6 10) the present study was undertaken to gain knowledge of the acute effects of intravenous administration of the drug on blood pressure and renal function

Material and methods

Eight patients in hospital with untreated benign essential hypertension participated in this study None of them was in congestive heart failure The patients are listed in table I Indwelling vascular catheters were used for blood sampling pressure recordings and infusions Blood pressures were recorded by an Elema strain gauge manometer Mean pressure was obtained by electrical integration Glomerular filtration rate (GFR) and renal plasma flow (RPF) were estimated as the clearances of inulin and para aminohippuric acid (PAH) using continuous infusion technics and mid period arterial blood samples Indwelling bladder catheters were used for urine collection and the bladder was rinsed by means of 100 ml distilled water and air The analytical methods for inulin para aminohippuric acid and electrolytes as for determinations of osmolarity in serum and urine used at this laboratory have been reported before (1)

Procedure

The studies were performed in the morning with the patients in the recumbent position Their hypertension was untreated and no pre medication was given The patients were in the fasting state but were allowed water ad libitum before the study Attempts were made to increase the water intake the hour before the experiment and to maintain a constant hydration during the experiment by giving the subjects as much water to drink as the excreted volume After the priming dose of inulin and PAH the patients

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TABLE I Mean values of two 20 min control periods for each patient and mean values of all control observations of each parameter with standard deviations (S D)

Patient Sex age	RPF (ml/min)	GFR (ml/min)	V (ml/min)	C_{osm} (ml/min)	C_{H_2O} (ml/min)	U_{Na} (μEq/min)	U_K (μEq/min)	BP_{mean} (mm Hg)
1 C A H ♂ 58	456	111	3 5	4 0	−0 5	306	59	131
2 T Z ♂ 61	329	68	2 6	2 4	+0 2	150	52	117
3 P O E ♂ 40	917	156	2 6	4 5	−1 9	179	57	126
4 A S ♂ 53	541	141	8 7	—	—	273	109	133
5 G A ♂ 63	434	112	2 6	3 7	−1 1	154	38	155
6 K S ♀ 59	478	79	2 2	2 3	−0 1	60	34	124
7 M A ♀ 37	472	111	7 8	2 2	+5 6	118	44	184
8 I V ♂ 51	263	72	5 3	3 7	+1 6	211	53	127
Mean ± S D	486± 195	106± 10	4 4± 2 6	3 3± 0 9	+0 5± 2 5	181± 66	56± 23	137± 22

Abbreviations RPF = renal plasma flow GFR glomerular filtration rate V urine flow C_{osm} = osmolar clearance C_{H_2O} = free water clearance U_{Na} = sodium excretion U_K — potassium excretion and BP_{mean} mean arterial pressure

were allowed to rest for at least half an hour for equilibration of blood concentrations of these substances Then five consecutive clearance periods each of 20 minutes were run The initial two periods were considered control observations During the first minute of the third period 0 5 mg ethacrynic acid per kg body weight was given in intravenous injection (50 mg ethacrynic acid was dis solved in 10 ml aqua dest)

TABLE II Effect of ethacrynic acid on renal hemodynamics water solute electrolyte excretion and

Period	RPF (ml/min)	GFR (ml/min)	V (ml/min)	C_{osm} (ml/min)
Control	486 (263−917)	106 (68−156)	4 4 (2 2−8 7)	3 3 (2 2−4 5)
0−20 min	441 (240−771)	98 (73−125)	18 5 (13 9 22 1)	17 3 (14 4−20 5)
20−40 min	423 (275−783)	88 (65−122)	18 2 (13 5−22 4)	16 9 (12 0 20 4)
40−60 min	415 (257−694)	82 (62−109)	11 2 (6 6−15 1)	10 8 (6 0 12 9)

Abbreviations see table I

The following parameters were determined in every period heart rate systolic di astolic and mean brachial arterial blood pressure (BP_{mean}) (blood pressures were recorded twice a period) GFR, RPF, urine flow (V) sodium excretion (U_{Na}) potassium excretion (U_K) osmolar clearance (C_{osm}) and free water clearance (C_{H_2O}) Free water clearance was calculated as $C_{H_2O} = V - C_{osm}$

Results

The results are given in the tables Table I shows the mean values of the two 20 min control periods for each patient and the mean values of all control observations with standard devia tions (S D) In table II the mean values for the different parameters in the different periods are recorded, and table III shows the differences from the control values of each observation period (given as a percentage)

Serum osmolarity

No consistent change in serum osmolarity was found (figures not presented)

Water and solute excretion

There was a rapid and marked increase in urine flow in all patients after ad ministration of the drug The urine flow increased from a mean of 4 4 ml per min to a mean of 18 5 ml per min in the first observation period In the second observation period the urine flow was about the same but diminished in the third observation period Osmolar clearance changed similarly so that no consistent change was produced in free water clearance

Electrolyte excretion

Sodium excretion increased more than 11 fold In the first two experimental periods the sodium excretion rate was about the same (mean 1,758 μEq per min) but decreased in the last period (1,060 μEq per min)

Potassium excretion rose far less than sodium excretion and increased on an average of 2 7-fold The excretion rate was the same in the first two observation periods (mean 140 μEq per min) but fell in the third observation period The ratio of urinary sodium to potassium in the control periods was 3 2 and in the three observation periods 12 3 12 7 and 10 5 respectively

Renal hemodynamics

After the drug injection there was a slight decrease in the figures of GFR and RPF in all but one patient The

blood pressure The mean values and range for each parameter in the different periods are recorded

C_{H_2O} (ml/min)	U_{Na} (μEq/min)	U_K (μEq/min)	BP_{mean} (mm Hg)
0 5 (−0 6− +0 6)	181 (60−306)	56 (34−109)	137 (117−184)
0 6 (−0 7− +3 0)	1 721 (1 160−2 261)	140 (83−206)	146 (104−200)
0 8 (0 1− +2 3)	1 786 (1 200−2 262)	140 (92−206)	141 (111−185)
0 1 (−1 4− +0 6	1 060 (544−1 429)	101 (57−143)	143 (103−205)

TABLE I Mean values of two 20-min control periods for each patient, and mean values of all control observations of each parameter with standard deviations (S D)

Patient Sex, age	RPF (ml/min)	GFR (ml/min)	V (ml/min)	C_{osm} (ml/min)	C_{H_2O} (ml/min)	U_{Na} (μEq/min)	U_K (μEq/min)	BP_{mean} (mm Hg)
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3 P.O.E. ♀ 40	917	156	2.6	4.5	−1.9	179	57	126
4 A.S. ♀ 53	541	141	8.7	—	—	273	109	133
5 G.A. ♂ 63	434	112	2.6	3.7	−1.1	154	38	155
6 K.S. ♀ 59	478	79	2.2	2.3	−0.1	60	34	124
7 M.A. ♀ 37	472	111	7.8	2.2	−5.6	118	44	184
8 I.N. ♀ 51	263	72	5.3	3.7	−1.6	211	53	127
Mean ± S D	486 ± 195	106 ± 10	4.4 ± 2.6	3.3 ± 0.9	−0.5 ± 2.5	181 ± 66	56 ± 23	137 ± 22

Abbreviations RPF = renal plasma flow GFR = glomerular filtration rate V = urine flow C_{osm} = osmolar clearance C_{H_2O} = free water clearance U_{Na} = sodium excretion U_K = potassium excretion and BP_{mean} = mean arterial pressure

were allowed to rest for at least half an hour for equilibration of blood concentrations of these substances. Then five consecutive clearance periods, each of 20 minutes were run The initial two periods were considered control observations During the first minute of the third period 0.5 mg ethacrynic acid per kg body weight was given in intravenous injection (50 mg ethacrynic acid was dissolved in 10 ml aqua dest.)

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Period	RPF (ml/min)	GFR (ml/min)	V (ml/min)	C_{osm} (ml/min)
Control	486 (263—917)	106 (68—156)	4.4 (2.2—8.7)	3.3 (2.2—4.5)
0—20 min	441 (240—771)	98 (73—125)	18.5 (13.9—22.1)	17.3 (14.4—20.5)
20—40 min	423 (275—783)	88 (65—122)	18.2 (13.5—22.4)	16.9 (12.0—20.4)
40—60 min	415 (257—694)	82 (62—109)	11.2 (6.6—15.1)	10.8 (6.0—12.9)

Abbreviations, see table I

The following parameters were determined in every period heart rate, systolic diastolic and mean brachial arterial blood pressure (BP_{mean}) (blood pressures were recorded twice a period) GFR RPF urine flow (V) sodium excretion (U_{Na}) potassium excretion (U_K) osmolar clearance (C_{osm}) and free water clearance (C_{H_2O}) Free water clearance was calculated as $C_{H_2O} = V - C_{osm}$

Results

The results are given in the tables Table I shows the mean values of the two 20 min control periods for each patient and the mean values of all control observations with standard deviations (S D) In table II the mean values for the different parameters in the different periods are recorded, and table III shows the differences from the control values of each observation period (given as a percentage)

Serum osmolarity

No consistent change in serum osmolarity was found (figures not presented)

Water and solute excretion

There was a rapid and marked increase in urine flow in all patients after administration of the drug The urine flow increased from a mean of 4 4 ml per min to a mean of 18 5 ml per min in the first observation period In the second observation period the urine flow was about the same but diminished in the third observation period Osmolar clearance changed similarly so that no consistent change was produced in free water clearance

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After the drug injection there was a slight decrease in the figures of GFR and RPF in all but one patient The

blood pressure The mean values and range for each parameter in the different periods are recorded

C_{H_2O} (ml/min)	U_{Na} (μEq/min)	U_K (μEq/min)	BP_{mean} (mm Hg)
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0 6 (−0 7− +3 0)	1 721 (1 160−2 261)	140 (83−206)	146 (104−200)
0 8 (0 1− +2 3)	1 786 (1 200−2 262)	140 (92−206)	141 (111−185)
0 1 (−1 4− +0 6	1 060 (544−1 429)	101 (57−143)	143 (108−205)

TABLE III Effect of ethacrynic acid on renal hemodynamics water solute electrolyte excretion and blood pressure. The deviations from mean control values in the different observation periods are given in per cent The mean differences and the S D of the mean differences are recorded

	First obs period	Second obs period		Third obs period	
Period	Diff %	Diff %	P	Diff %	P
RPF	−6 6±7 4	−10 8±4 8		−12 0±3 9	p< 0 05
GFR	−4 6∓6 5	−14 3±5 6	p< 0 05	−20 0±4 0	p< 0 01
V	429±86	424±90		214±47	
C_{osm}	474±56	446±55		243±31	
U_{Na}	1 059±260	1 080±183		548±87	
U_K	165±21	167±28		95±21	
BP_{mean}	+6 3±2 9	+4 2±1 9		+2 5±2 6	

Abbreviations see table I

decrease of RPF was not significant in the first two observation periods, but probably significant in the last period ($p < 0\ 05$) The decrease of GFR was not significant *in the first observation* period In the second observation period the decrease of GFR was probably significant and in the last period it was significant ($p < 0\ 01$)

Blood pressure and heart rate

There was no consistent change in heart rate or in blood pressure (only mean arterial blood pressures are reported in the tables

Side reactions

Three subjects felt transient pain in the arm during the injection Otherwise no side reactions were encountered

Discussion

Our study has shown that 0 5 mg ethacrynic acid per kg body weight, given intravenously to patients with essential hypertension, has a rapid and powerful diuretic action Cannon et al (3 a) giving larger doses noted an even more pronounced diuretic response in one patient with cardiac decompensation and in two normal subjects

The osmolar clearance increased in the same way as the urine flow under the hydration conditions used Consequently the free water clearance was not significantly altered Goldberg et al (4) have shown that free water clearance varies with the experimental conditions In maximal hydrated subjects the drug caused diminished C_{H_2O} In hydropenic subjects the drug caused reduced capacity to concentrate the urine They have put forward the hypothesis that the drug has its main site of action in the ascending limb of Henle s loop At that site *sodium chloride* is *reabsorbed* in excess of water Inhibition of reabsorption of sodium in the loop would prevent the accumulation of hypertonic medul

lary solute and thus prevent the counter current multiplier system from functioning

The diuresis was associated with massive natriuresis The peak rates of natriuresis corresponded to 8 6—19 8 % of the estimated filtered load of sodium These figures are in the same range as those given by Nash et al (9) The kaliuresis increased far less than the natriuresis and our figures are in the same range as those of Cannon et al (3 a)

There was a slight decrease of RPF and GFR in our series The decrement was most marked in the last observation period where RPF showed a mean decrease of 12 % (probably significant) and GFR a mean decrease of 20 % (significant) Similar results have been reported by Nash et al (9) and Brest et al (2) Cannon et al (3 a) noted a reduction of about 30 % of RPF in two normal subjects In two cardiac and two cirrhotic patients with fluid retention however RPF rose after intravenous injection of ethacrynic acid

Arterial blood pressure and heart rate was unaltered in this study Nash et al (9) studied the hemodynamic effects of ethacrynic acid given intravenously in 13 patients They found no significant changes in systemic arterial blood pressure mean right atrial pressure heart rate cardiac output or total systemic resistance Dollery et al (3 b) who studied the acute effect of ethacrynic acid orally in seven hypertensive patients noted a considerable fall in two of them but both were on hypotensive drugs Long term studies in patients with hypertension (3 a 3 b 6) have shown that the drug has a hypotensive effect at least in some patients

Our study has shown that the drug has a quick and potent diuretic action, which makes it suitable for the treatment of pulmonary edema It had no unfavourable effect on blood pressure from this point of view The drug does not cause great reduction in glomerular filtration rate or renal plasma flow

Summary

The effect of ethacrynic acid intravenously administered was studied in 8 patients with arterial hypertension without cardiac decompensation Renal hemodynamics water and electrolyte excretion blood pressure and heart rate were studied for five periods of 20 min The drug was given after two control periods (0 5 mg/kg body weight) A marked increase in urine flow was achieved Natriuresis increased on an average of about 11 fold Kaliuresis increased far less (on an average of 2 6 fold)

Renal plasma flow and glomerular filtration rate decreased slightly The drug had no effect on blood pressure or heart rate

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Blood Coagulation and Plasma Fibrinolysis in Geriatric Patients with Decreased Liver Function

By

PREBEN OLLENDORFF, JORGEN RASMUSSEN and TAGE ASTRUP

The occurrence of defective blood coagulation in patients with liver diseases is well known The deficiency may be due to a decrease in the concentration of factors of the coagulation system as first observed by Quick et al (28) in patients with jaundice Increased fibrinolytic activity in blood was first reported in patients with liver cirrhosis by Goodpasture (15) These observations have repeatedly been confirmed by later authors If the clotting of blood is delayed and the fibrinolytic activity is increased, formation and deposition of fibrin becomes complicated Though it is difficult to evaluate the contribution of each defect a haemorrhagic tendency can certainly be anticipated This is in accordance with a number of clinical observations Lately reports have appeared which indicate that clotting defects probably are not so common in patients with liver cirrhosis as was formerly believed Most of the fibrinolytic studies until now appear to have been performed on blood from severely affected patients

Hedenberg and Korsan Bengtsen (18), including mild cases in their studies, observed that only a few of them gave abnormal coagulation tests Several of the severe cases also gave normal values Similarly Kupfer et al (22) assaying coagulation factors and fibrinolytic activity in twenty five severely ill patients with the whole blood clot lysis method, found all patients to be in the normal range of fibrinolytic activity Reports suggesting that fibrinolysis in liver cirrhosis is secondary to disseminated coagulation led Johansson (20) to try the effect of heparin in a patient who was severly ill though with no demonstrable fibrinolytic activity A rise occurred in platelet number and in the level of several blood coagulation components Fletcher et al (11) suggested that increased fibrinolysis in the cirrhotic patient could be due to a failure of a

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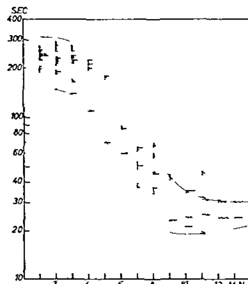


Fig 1 Thromboplastin activation test (TAT) in 58 patients with liver cirrhosis Abscissa Minutes after recalcification of the platelet rich diluted plasma sample Ordinate Coagulation time in seconds of 0 1 ml of the platelet poor substrate plasma after addition of 0 1 ml of the incubation mixture and recalcification logarithm c) The normal range is indicated by the two curves

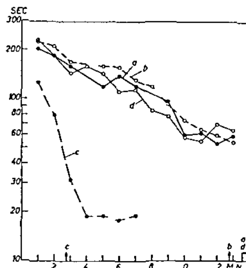


Fig 2 TAT on plasma from a patient (D J) with prolonged prothrombin time (Quick index 53 P P value 29 %) normal fibrinolytic activity and deficiency of Factor IX (Christmas factor) Curve a Thromboplastin activation in the patient s platelet rich plasma Curve b TAT after addition of 20 % adsorbed plasma Curve c TAT after addition of 20 % normal serum Curve d TAT after addition of 20 % normal serum adsorbed by $BaSO_4$ and heated to 56 The arrows indicate the clotting times of the respective incubation mixtures

hepatic clearance mechanism for plasminogen activator They drew attention to the fact that despite the efforts of many investigators these liver disorders are poorly understood and that conventional coagulation assays sometimes fail to provide an adequate explanation for the clinical findings It is apparent that the interrelation between liver cirrhosis and defects of coagulation is not as simple as was formerly believed and that detailed studies are needed to clarify the problem It is the purpose of the present study to assist in this clarification by reporting some coagulation data and fibrinolytic assays from a group of patients with the mild degrees of liver cirrhosis seen in geriatric patients with decreased liver function

Material and methods

Blood samples were collected from patients at the Geriatric Unit of the Old People s Town or from the Medical Department E of the Frederiksberg Hospital The material consisted of 58 patients in whom liver cirrhosis had been suggested by a positive Takata Ara flocculation test or by the retention of more than 10 % of sulfobromophtalein sodium 45 minutes after intravenous injection of 5 mg per kilogram of body weight Of the patients 21 were men and 37 women Their ages ranged from 65 to 95 years None of them showed a severe haemorrhagic disorder Most patients were only mildly affected and had not been admitted to the hospital for their liver disease Our group of patients represents the mild cases often seen in elderly people

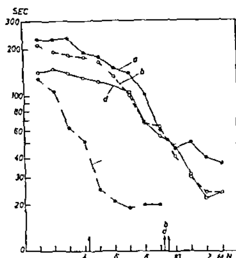


Fig 3 TAT on plasma from a patient (NPL) with normal prothrombin time and deficiency of Factor IX The fibrinolytic activity was not estimated Labelling as in fig 2

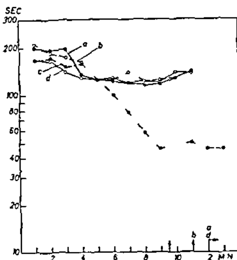


Fig 4 TAT on plasma from a patient (LPH) with a much prolonged prothrombin time (Quick index 37) deficiency in Factor IX and increased fibrinolytic activity Labelling as in fig 2

Blood was drawn early in the morning in citrate by silicone technique and kept on ice until within an hour the plasma could be prepared and the cougulation tests performed For the fibrinolytic estimations the platelet poor plasma was kept on ice and later stored at — 20 until several samples could be assayed simultaneously Separate investigations had shown that the spontaneous activity was stable under these conditions As a control one or more frozen normal samples were essayed simultaneously with the samples from the patients These normal samples however mostly originated from much younger persons (table IV)

The activity of the plasma thromboplastin system was studied with the thromboplastin activation test (TAT) of Astrup and Ollendorff (4) In this method which is sensitive to early stages of the activation process the addition of serum is avoided in order to exclude an enhancing effect of factors not present in genuine plasma When a defective thromboplastin system was encountered this was further investigated by studying the correcting effects of small amounts of bovine plasma adsorbed by BaSO of normal aged human serum and of serum which had been adsorbed by BaSO and heated at 56 for 30 minutes

The TAT reflects the adequacy of the plasma factors necessary for thromboplastin formation provided that the levels of prothrombin proaccelerin (Factor V) and Factor X are not severely reduced This was checked by the one-stage prothrombin time test of Quick in which the combined effect of all factors in the prothrombin system (viz prothrombin F V F VII and F X) is measured (using an aqueous human brain extract (Owren and Aas (75)) as thromboplastin source) As usual the result was calculated as the ratio between the clotting time of a normal control and the clotting time of the patient s plasma and as expressed in percentages as a Quick index though this method of calculation can be criticized (Astrup (5))

Fibrinolytic activity was assayed on normal fibrin plates containing 0 12 % bovine fibrinogen rich in plasminogen and clotted with bovine thrombin (Leo Pharmaceuticals Copenhagen) Protease activity was assayed on fibrin plates heated for 45 minutes at 85 Serial dilutions of plasma were applied directly on the plates Euglobulins were

TABLE I 11 patients with thromboplastic defects The fibrinolytic activity on normal and heated fibrin plates is expressed as the product of the longest and the shortest diameter of the digested area (average of triplicates)

Patient	Sex	Age	Normal fibrin eugl	Normal fibrin plasma	Heated fibrin eugl	Heated fibrin plasma	Quick index
DJ	F	75	30	0	4	0	53
ER	F	72	47	0	31	0	71
KN	F	69	36	0	27	0	76
SJ	F	90	136	60	36	34	87
AK	F	85	121	36	36	25	81
RCH	M	80	100	4	42	0	54
LPH	M	76	114	73	30	36	32
LM	F	78	255	0	16	0	75
LS	F	79	16	0	25	0	100
MF	F	76	36	0	4	4	78
NPL	M	81	—	—	—	—	90
Average ($\bar{x}$)		78	89	17	25	10	72
Range		69—90	16—255	0—73	4—42	0—36	32—100
S D		—	72	28	10	15	20
Number (n)		11	10	10	10	10	11

— = not examined

S D is estimated as $\sqrt{\frac{\Sigma x^2 - \bar{x}\Sigma x}{n-1}}$

x = the individual estimation

$\bar{x}$ = the arithmetic average

precipitated at pH 5 9 (Astrup and Rasmussen (1)) and the dilutions prepared and assayed as before (Astrup et al (2))

Results

The distribution of the data obtained in the thromboplastin activation test (TAT) is shown in fig 1, which includes the limits of the range of normal samples as obtained previously (4)

A defect in the plasma thromboplastic system was found in 11 of the 58 patients In 10 of them supplementary experiments were performed The defective TAT could be corrected by addition of normal, aged serum but not by adsorbed bovine plasma or by adsorbed, heated normal serum This would suggest a defect in Factor IX (Christmas factor, PTC) or Factor X (Stuart Prower factor) Fig 2 and fig 3 show typical cases

From the prothrombin estimations it is seen that the prothrombin system was affected in most of the 10 patients but in only one patient so much that the defective TAT could in part be caused by a low prothrombin content (fig 4)

It was of particular interest that in 5 out of the 10 patients in whom a decreased thromboplastin activity was ob

TABLE II 12 patients with normal thromboplastic activity and increased fibrinolytic activity (see text)

Patient	Sex	Age	Normal fibrin		Heated fibrin		Quick index
			eugl	plasma	eugl	plasma	
CA	F	79	127	35	53	4	60
KN	F	80	121	4	49	4	94
AO	F	87	144	0	81	0	100
DH	F	90	225	64	49	49	75
MM	M	94	100	4	36	0	60
IL	F	71	121	0	25	0	98
EA	M	87	140	4	36	4	87
KE	F	87	225	0	36	0	64
PP	F	86	49	47	29	0	95
HM	M	73	121	4	4	0	100
ADP	F	71	121	0	27	0	100
RM	M	79	127	4	42	4	100
Average ($\bar{x}$)		82	135	14	39	5	86
Range		71—94	(49)—225	0—64	4—81	0—49	60—100
S D		—	49	22	19	14	17
Number (n)		12	12	12	12	12	12

TABLE III 33 patients with normal thromboplastic and normal fibrinolytic activity

	Age	Normal fibrin		Heated fibrin		Quick index
		eugl	plasma	eugl	plasma	
No of patients (n)	33	33	33	33	33	31
Average ($\bar{x}$)	80	40	0	19	0	90
Range	65—95	0—81	0—4	0—49	0—4	50—112
S D	—	26	—	14	—	18

TABLE IV The 15 plasma samples which were used as controls for the fibrinolytic estimations

	Normal fibrin		Heated fibrin	
	eugl	plasma	eugl	plasma
No of patients (n)	15	15	15	15
Average ($\bar{x}$)	52	0	24	0
Range	0—97	0—4	0—49	0
S D	23	—	14	

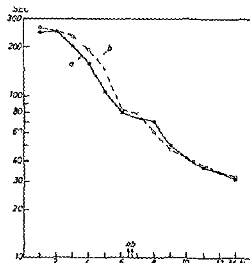


Fig 5 TAT on plasma from a patient with increased fibrinolytic activity Curve a before and curve b after incubation of the plasma for 90 minutes at 37

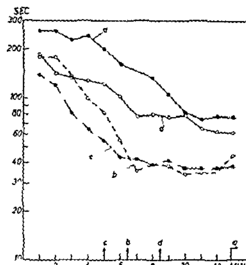


Fig 6 TAT on plasma from a 54 year old male patient possibly lacking Factor XI (PTA) Quick index was 75 Fibrinolytic activity not estimated Labelling as in fig 2

served, *an increase in fibrinolytic activity* was found (table I) The effect on heated fibrin demonstrates formation *of plasmin in the samples*

Slightly increased fibrinolytic activity was observed in another 12 patients, in whom no thromboplastin defect could be demonstrated (table II) making a total of 17 with increased fibrinolytic activity out of 55 investigated Fewer of the samples in table II showed activity in plasma by direct application than in the group where a thromboplastic defect was present (table I)

In the remaining 35 patients there was normal thromboplastin formation, and in 33 of these patients tested for fibrinolytic activity this was within its normal range (table III)

Because of the *low* fibrinolytic activity it could not be assumed that the thromboplastic defect could have been caused by an effect *of the fibrinolytic enzyme on* one or more of the factors of the thromboplastic system However in order to exclude this possibility, 25 plasma samples *chosen at random were placed in* siliconed tubes in a waterbath at 37° for 90 minutes The TAT was then performed and the result compared with the results of the test on the same plasma before incubation In none of the samples was a decrease in thromboplastin activity found Fig 5 shows an example of incubation of a sample from a patient with increased fibrinolytic activity

A patient who was not included in the above material as he belonged to a younger age group, will be mentioned here because he had a plasma thromboplastic defect not previously described in connection with liver cirrhosis The patient was severely affected and died in hepatic coma 15 days after the investigation The TAT showed defective *thromboplastin activation, which could*

be corrected by adsorbed bovine plasma, as well as by serum whereas adsorbed heated serum had only a slight effect (fig 6) Since adsorbed heated serum contains of known clotting factors, only Factor XII (Hageman factor) and traces of Factor XI (PTA), the results suggest that the patient s plasma lacks Factor XI Unfortunately, the fibrinolytic system was not studied in this patient

Discussion

In cirrhosis of the liver the prothrombin system including F V (proaccelerin) F VII (proconvertin) and F X (Stuart Prower factor), as assayed by the one stage prothrombin time test, is often affected Thus Broicher et al (8) observed that in 260 patients with varying types of liver disease F V F VII and prothrombin were the most seriously reduced followed by Factor X Rabiner and Spaet (31) found that many patients suspected to have liver disease despite negative liver function tests, had low Factor X content in their serum Rapaport et al (32) found a pronounced decrease in F VII and F IX in some patients with only a moderately prolonged Quick time Geill (14) using the thrombin generation test demonstrated reduced thrombin formation in 13 patients with liver cirrhosis Facchini et al (10 Rabiner (29) Rabiner and Schulman (30) and Cocchen and Mighori (9) also observed a serum defect in the thromboplastin generation test All these authors found a very high incidence of coagulation defects in patients with liver cirrhosis

Kupfer et al (22 studied the concentration of individual clotting factors (except Factor XI and Factor XII) in 25 patients with severe liver cirrhosis They found Factor VII low in 21, Factor V low in 16 and low Factor X and Factor IX content in about half of the patients Recently, Hallen and Nilsson (17) studied 20 patients with varying degrees of cirrhosis Owren s P P test gave a decreased value in 16 of the patients low Factor V values were found in 17 patients, and a decrease in Christmas factor (F IX) was found in 8 patients Plasma antithrombin was increased in 66 % of the patients They also tested the fibrinolytic activity in plasma on normal fibrin plates and found a slight increase in 5 cases

Weawer et al (36) found F VII to be the factor most decreased in liver diseases especially in cirrhosis but they did not assay for F X or for F IX of the plasma thromboplastin system

It is of interest that coagulation defects are also seen in patients with viral hepatitis (Rabiner (29) Cocchen and Mighori (9)) in that there is general agreement that the fibrinolytic activity in blood is not increased in these cases cf de Nicola and Soardi (23)

Hedenberg and Korsan Bengtsen (18) using a modified thromboplastin generation test and a modified thrombin generation test found a thromboplastic defect in only one of 22 mild histologically verified cases of liver cirrhosis whereas 5 of 12 severe cases had a thromboplastic defect A prolonged Quick time was found in one patient in the mildly affected group whereas 6 had a lowered P P value Among the 12 severely affected patients 6 had a prolonged Quick time and 7 a lowered

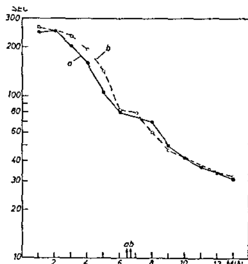


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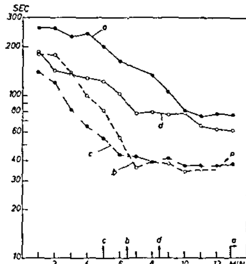


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lytic activity in blood and the severity of liver disease (for references see de Nicola and Soardi (23)) No such correlation was observed by Kwaan et al (21) who, however found that adrenaline caused a much larger increase of fibrinolytic activity in blood of patients with liver cirrhosis than in normal persons This might be correlated with the observation by Weiner (37) and Fletcher et al (11) that patients with liver cirrhosis respond with production of more fibrinolytic activity after administration of nicotinic acid than normal persons Fletcher et al also reported a lower plasma clearance rate of plasminogen activator in patients with liver cirrhosis than in normal persons

A decreased inhibition of fibrinolysis could in part be responsible for the increased activity recorded in patients with liver cirrhosis Our results show that activity was frequently observed in the diluted plasma, even if no activity was found in the undiluted plasma Phillips and Skrodelis (26) have reported on two patients with liver cirrhosis and haemorrhagic diathesis with a decreased level in the blood of an inhibitor of fibrinolysis When a larger group of patients with liver cirrhosis and haemorrhagic diathesis was compared with a group of milder cases and with a series of normal controls, the latter two groups showed no spontaneous fibrinolytic activity whereas many patients in the haemorrhagic group had spontaneous fibrinolytic activity The authors reportedly used serum in their studies of fibrinolytic activity Purcell and Phillips (27)

In comparison we found by a sensitive technique an increased fibrinolytic activity in 31 % of the patients with mild liver cirrhosis The source of the increased fibrinolytic activity is unknown, though it is tempting to correlate the activity in blood with the high content of plasminogen activator found in the liver in cases of cirrhosis by Astrup et al (3) This is in contrast to the nearly total absence of fibrinolytic activity in the normal liver, although a slight increase with age was observed

There are reports suggesting that the fibrinolytic activity in blood in liver cirrhosis can prevent or decrease clot formation Spitzer et al (35) noticed the absence of pulmonary embolism in a series of patients with liver cirrhosis Confirming these observations, Geill (12, 13), in an autopsy material, found a low incidence of thromboembolism in cases of liver cirrhosis Myocardial infarctions are less frequent in patients with liver cirrhosis (Grant et al (16), Howell and Manion (19)) It is known that patients with liver cirrhosis show an increased sensitivity to anticoagulant drugs, cf the discussion by Sherlock (34) The complexity of the mechanism alluded to in such studies, however is demonstrated by the finding of fresh venous thrombi in the patient with liver cirrhosis and haemorrhagic diathesis *described by Beaumont et al (6)* Bergstrom et al (7) studied a patient with cirrhosis of the liver with increased, but varying fibrinolytic activity in the blood Because of a decrease in some of the clotting factors, they suggested that the increase in fibrinolytic activity was secondary to hypercoagulability Johansson (20) believed that the severe haemorrhagic diathesis in his patient was

due to a decrease in the clotting factors following intravascular coagulation No increase in the fibrinolytic activity could be demonstrated in his case

In the present series no patients had excessive fibrinolytic activity in the blood There were more patients with enhanced fibrinolytic activity than with a defective blood clotting system There was an accumulation of patients with increased fibrinolytic activity among the patients with defective blood coagulation This preponderance was not only numerical (5 cases with fibrinolytic activity out of 10 cases with delayed TAT, against 12 out of 45 with normal TAT) but was also quantitative since in all but one of the cases with defective thromboplastin formation, there was fibrinolytic activity in plasma as well as in the euglobulin fraction

Summary

Blood coagulation and fibrinolysis have been assayed in plasma from 58 elderly patients in whom laboratory test had indicated decreased liver function suggesting cirrhosis of the liver In 10 of the patients there was abnormal thromboplastin activation In 17 patients increased fibrinolysis could be detected in the euglobulin fraction on normal and heated fibrin plates In some of these plasma dilutions also showed fibrinolytic activity Among the 10 patients with thromboplastic defects 5 had increased fibrinolytic activity The results are compared with previous reports on more advanced cases Apparently, there is little change in blood coagulation and fibrinolysis in milder cases of liver cirrhosis

Acknowledgements

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Familial Incidence of Thyroid Disease and Anamnestic Incidence of Pubertal Struma in 449 Consecutive Struma Patients

By

PETER HEIMANN

Despite our increased knowledge of the function and pathophysiology of the thyroid gland the aetiology of most thyroid diseases is still unknown According to Klein (3) a familial incidence of thyroid disease is found in about one third of patients with all types of diseases of the thyroid gland The disposition to thyroid disease appears to be hereditary, while it may also originate in a number of factors such as iodine deficiency administration of strumogenes endogenous adjustment processes (as during puberty, pregnancy and climacterium) and physical and mental stresses The heredity of thyroid disease has interested a number of authors, among whom Bartels, (1) Fraser (2) Stanbury and McGirr (7), Sorensen (8) and Vague et al (9)

In principle one can either make a thorough survey of the hereditary incidence of struma by tracing and examining all members of a family, or one can determine the incidence of familial thyroid disease by studying the conditions in a larger material In the latter case, however, it is impossible to examine all members of the family One can merely estimate the occurrence probably as a minimum incidence

One of the questions in this study was how great is the incidence of familial thyroid disease in patients with atoxic and toxic struma? The question is of interest for illustrating the conditions in western Sweden The nearness to the sea, the relatively high iodine content in the water and the abundance of saltwater fish confirm assumption that there is no deficiency of iodine in the area so that endemic struma is rare One may expect that the hereditary factor has a greater role in a region where one of the most important exogenous factor (iodine deficiency) is less significant

The other question which stimulated this study derives from investigations made by Nilsson at the Gothenburg

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TABLE I Age groups sex and diagnostic groups Benign thyroid disease occurred in 435 of the patients 145 of whom were in the below 40 age group (33%) 195 in the 40—60 age group (45%) and 95 in the above 60 age group (22%)

Diagnosis	Below 40 years of age		40—60 years of age		Above 60 years of age		All age groups		
	Male	Female	Male	Female	Male	Female	Male	Female	Total
Atoxic struma	12	84	11	93	9	47	32	224	256
Toxic struma	4	34	7	48	6	15	17	97	114
Thyroiditis	0	11	3	33	2	16	5	60	65
Malignant struma	0	0	0	6	2	6	2	12	14
Total	16	129	21	180	19	84	56	393	449
	145		201		103				

Children's Hospital in collaboration with Doniach and Persson (4 5) The latter investigations showed that a surprisingly large proportion of the struma cases among children aged 10—16 in the hospital were chronic thyroiditis

Nilsson and Persson after fine needle puncture of 48 children with struma found chronic thyroiditis in 27 of them Investigations of the occurrence of antibodies to thyroid antigen and of disturbances of the I^{131} metabolism also showed the incidence of autoimmune thyroiditis (40%) to be strikingly high among children with struma in the Gothenburg area Forty five per cent of the children had colloid struma

The question that these conditions gave rise to was what happens to these children with chronic thyroiditis and *colloid struma? What happens to their* struma in adulthood how many of them develop hypothyroidism? Naturally the children are carefully followed up but many years must elapse before one can get an answer to this question At the same time many of them are treated with l thyroxin in substitution dose so that the prognosis is naturally not the same as if they had been untreated One may therefore consider attacking the problem from another angle by attempting to estimate the incidence of pubertal struma restrospectively in the history of patients with thyroid disease

It would naturally be of interest to examine a series of patients with hypothyroidism I have no such material available however at the Surgical Clinic — with the exception of patients with postoperative thyroid hypofunction But it is also of interest to study the incidence of patients with pubertal struma among those with atoxic and toxic struma I have therefore combined this study with that of the incidence of *familial struma The linking together of* these two factors is of some interest also since Nilsson (6) considered he had evidence that children with autoimmune thyroiditis or colloid goiter often have a family history of thyroid disease

Material

The study comprises 449 consecutive cases of thyroid disease examined and treated by me in the Struma Section of Surgical Clinic II. The patients were treated during the period November 1 1960—October 31, 1964.

Cases assessed and treated by other physicians and merely sent to me for consultation or continued treatment (e.g. postoperative hypothyroidism) have been excluded. The patients included in the study are those assessed by me from the outset in a uniform manner and whose diagnoses are therefore comparable. All patients have been taken in consecutive order so as to avoid a selection which would distort the results.

The material has been divided into four diagnostic groups (atoxic struma, toxic struma, thyroiditis and malignant struma) and into three age groups. The sex distribution in these groupings is shown in table I. Of 449 patients with thyroid disease 57 % (256) had atoxic struma, 25 % (114) toxic struma, 14 % (65) thyroiditis and 3.1 % (14) malignant struma. An over representation of patients with chronic thyroiditis and malignant struma may be expected at a special section for thyroid diseases at a university clinic.

Some patients of course had to be excluded from the study — those who were ignorant of their relatives, patients with whom I lost contact and patients who died before the enquiry could be completed. Altogether 34 patients were excluded on these grounds (table II). A total of 415 patients (92 %) therefore remained, which must be considered to be a satisfactory number.

The comparatively small group of malignant struma is not included in the report of the results.

Method

A questionnaire was sent to 223 patients and 192 (46 %) were questioned by me personally during visits to the clinic at the time of the investigation. As regards heredity I did not limit my investigation to a general question but asked the patients about all

TABLE II. Uninvestigated patients by diagnosis and sex

Diagnosis	Male	Female	Total
Atoxic struma	2	21	23
Toxic struma	1	4	5
Thyroiditis	2	4	6
Total	5	29	34

relatives and the diseases they were known to have had — viz. parents, uncles and aunts, grandparents, brothers and sisters, children and cousins.

Only simple calculations of the percentage incidence have been carried out. In a study of this kind statistics can give only an indication of tendencies and must be judged in relation to the incompleteness of the questioning method. It was therefore not considered worth while to subject the material to detailed statistical analysis.

Results

Incidence of familial struma

A family incidence of struma was found in 168 patients (42 %). The distribution among the diagnostic groups was atoxic 97 patients (58 % of all patients with struma in the family), toxic struma 52 patients (31 %) and thyroiditis 19 patients (11 %). The distribution is almost identical to that for the entire material.

The incidence of family struma in the various diagnostic and age groups is shown in table III. There is no significant difference between the four diagnostic groups. Family struma was present in 40—50 % of these patients, a figure which is higher than that usually quoted previously of 30—33 %.

TABLE III Percentage of familial struma by diagnostic and age groups There were no significant differences

Diagnosis	<40 years	40—60 years	>60 years	Total
Atoxic struma	41	48	30	41 ± 3
Toxic struma	46	48	50	47 ± 5
Thyroiditis	36	36	20	32 ± 6
Total	42	46	62	42 ± 2

TABLE IV Incidence of familial struma in relation to diagnosis and number of family members with struma There is no statistical difference between the various groups One may thus say that a family history of thyroid disease is equally common in patients with all types of benign struma

Diagnosis	No of patients studied	Thereof with familial struma	%	Struma in 2 members of family	%	Struma in 3 members of family	%	Struma in more than 3 members of family	%
Atoxic struma	233	97	42	60	26	17	7	20	9
Toxic struma	109	52	48	30	28	14	13	8	7
Thyroiditis	59	19	32	15	25	3	5	1	2
Total	401	168	42	105	26	34	9	29	7

Table IV shows the incidence of families whith 2, 3 or more members suffering from struma The maximum is 8 members with thyroid disease in the families of a few patients In 38 % of the patients with family occurrence of struma struma was reported in 3 or more members of the family This corresponds to an incidence of nearly 16 % in the entire material of benign thyroid disease That is to say that roughly every sixth patient with benign thyroid disease reported struma in 3 or more members of the family Only 2 familial members with definite thyroid disease were recorded in the case of 105 patients (26 %), i e every fourth patient In these cases it was generally the parents (especially the mother), siblings or children who had struma

Summary

A familial occurrence of thyroid disease was found in 42 % of patients with atoxic struma, toxic struma or thyroiditis For patients with toxic struma the figure was 47 %, with atoxic struma 41 % and with thyroiditis 32 % No difference was found between the sexes Familial struma was found rather more often in the case of the two younger age groups (45 %) than of patients above 60 years of age (32 %)

TABLE V Percentage of pubertal struma by diagnostic and age groups. Pubertal struma appears to be more common among patients with atoxic struma than with toxic struma or thyroiditis. This difference is significant

Diagnosis	<40 years	40—60 years	>60 years	Total
Atoxic struma	43	16	30	24 ± 3
Toxic struma	15	6	15	10 ± 3
Thyroiditis	36	9	0	12 ± 4
Total	34 ± 4	11 ± 2	9 ± 3	18.5 ± 2

Incidence of pubertal struma

Definite pubertal struma was traced in 74 of the patients whith benign struma (18.5 %) — 56 in the atoxic struma, 11 in the toxic struma and 7 in the thyroiditis group. Only 4 of the patients with pubertal struma were male, 94 % being female. The percentages in different age and diagnostic groups are shown in table V. Pubertal struma is obviously rather more common in the history of patients with atoxic struma (especially at younger ages) than with toxic struma or thyroiditis. One can hardly ascribe significance to the higher incidence of pubertal struma in the age group below 40 years, since in all probability these patients have a clearer remembrance of their puberty. In point of fact one should assume the figures for this age group to be the most relevant. This would imply that pubertal struma is as common in patients with atoxic struma as with thyroiditis but significantly lower in patients with toxic struma.

Incidence of pubertal combined with familial struma

In the atoxic struma group pubertal combined with familial struma occurred in 29 patients (12 %). Familial struma was recorded in 52 % of patients with pubertal struma against 38 % of patients without demonstrable pubertal struma. The difference is significant. Conversely, pubertal struma occurred in 30 % of patients with familial struma against 20 % of patients without. This difference is not significant.

Corresponding calculations for patients with toxic struma show that 8 of 11 patients with pubertal struma report struma also in the family. On the other hand only 15 % of patients with family struma had had struma in puberty. Here again accordingly it is more common that patients with a history of pubertal struma also have struma in the family.

In the entire material 40 patients had both pubertal and familial struma (10 %), viz. 25 % of the patients with familial struma and 54 % of those with pubertal struma.

Discussion

As already pointed out, the retrospective method of study of the familial occurrence of a disease has certain shortcomings owing to sources of error such as incomplete information, undiagnosed

disease, and the difficulty of correctly assessing the patients statements The figures obtained in such a study must therefore be taken with considerable reserve I nevertheless think that they allow an acceptably approximate estimate of the tendencies

According to the literature, summarized by Klein (3), family struma occurs in roughly one-third of patients, whichever thyroid disease one examines In our material the figures are throughout higher and since they must be considered to be minimum figures in view of the sources of error referred to above,one may say rather that nearly half of all patients with thyroid disease appear to *have thyroid disease in the family* Thus a hereditary disposition to thyroid disease appears to have an important role in its contraction especially in the west-coast region of Sweden with its relatively ample supply of iodine and absence of endemic struma

Pubertal struma is found almost exclusively in girls It is also clear that pubertal struma is more common in the history of patients with atoxic than with toxic struma Here again the figures must be taken as minima The higher incidence in the younger age groups must be ascribed rather to the fact that these patients remember their puberty better

As regards the incidence both of pubertal struma and familial thyroid disease, the number of patients is comparatively small and the findings must be assessed with caution One may nevertheless definitely assert that a family history of thyroid disease is rather more common in patients who have had pubertal struma There was thyroid disease in the families of more than half of the patients with atoxic and pubertal struma

The comparative uncertainty of the reported figures is reason not to extend the analysis to other details

Mention may also be made of the considerably greater information one obtains from the patient by careful questioning than by the routine discussion of heredity which forms part of the normal recording of case histories

It would be desirable that this study should be supplemented by a similar study of primary hypothyroidism A closer relationship whould then probably be found with chronic thyroiditis during puberty or in the patient s general history

But there would appear already to be sufficient reasons for devoting great attention to patients with pubertal struma They should be closely followed and should possibly be given thyroid hormone during puberty, pregnancy and the climacteric

Summary

Of 449 consecutive patients with thyroid disease (atoxic struma, toxic struma, thyroiditis and malignant struma) 415 were questioned orally or in writing concerning the incidence of thyroid disease in their families and concerning struma in their own puberty

Forty two per cent of the 401 patients with benign thyroid disease had a familial incidence of thyroid disease No significant difference was found between

different age or diagnostic groups, nor between the sexes

Of patients with benign thyroid disease 18.5 % had had struma in puberty. Pubertal struma was more common in the history of patients with atoxic struma, and probably also of patients with thyroiditis than of patients with toxic struma. This is suggested by the figures for the youngest age group, which should be the most relevant in this context.

Familial as well as pubertal struma were reported by 10 % of the patients, viz. by 25 % of the patients with familial and 54 % of those with pubertal struma. The incidence of familial thyroid disease was significantly greater for patients with pubertal struma (the atoxic struma group) than without.

In conclusion this study — despite its defects — indicates that a hereditary disposition to thyroid disease plays an important role in the area of the study, where iodine deficiency and endemic struma are rare. About half of all patients with thyroid disease appear to have had a history of the disease in the family. Strikingly many (16 %) report the disease in more than three members of the family.

The combination of familial and pubertal struma would also appear to be fairly common.

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Migraine, Gastritis and Renal Papillary Necrosis

A Syndrome in Chronic Nonobstructive Pyelonephritis

By

Ó Olafsson[1], K R Gudmundsson and A Brekkan

Renal damage occurring in association with long abuse of phenacetin was first described in 1953 by Spuhler and Zollinger (20) Since then numerous papers on the subject have been published in Europe and the U S A In most of the European reports (7 8 9 12 14 15 17, 20 21 and others), phenacetin was primarily incriminated as the causal factor More recent reports from the U S A (3 18 19), however have emphasized that a majority of the affected persons had used compound preparations (phenacetin with acetyl salicylic acid, caffeine etc)

The renal disorders associated with habituation to phenacetin or phenacetin-containing compounds are chronic interstitial nephritis, papillary necrosis and chronic pyelonephritis Many writers have expressed the opinion that phenacetin in these cases gives rise to interstitial nephritis and the resultant diminution of the blood supply to the renal papillae causes papillary necrosis (20) Others stated that chronic interstitial nephritis ascribed to phenacetin abuse has many gross and microscopical features in common with true non-obstructive chronic pyelonephritis (2 a) Renal papillary necrosis however is almost always associated with heavy use of analgesics and rarely complicates such pyelonephritis (10)

The necrotic papillae may calcify or they may be shed and excreted in the urine The extent of the necrosis varies It may be well demarcated centrally in the papilla or more diffuse involving much of the papillary and medullary substance

Two main hypotheses exist concerning the basic mechanism of phenacetin induced renal papillary necrosis

a) Phenacetin produces toxic hypoxia in the kidneys leading to death of papillary tissue (22)

b) An auto-immune reaction to phenacetin or its metabolites involves the renal tissues with tubular damage as the main

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TABLE I The main analgesic preparations (containing phenacetin) available in Iceland

	Content of phenacetin	Other analgesic ingredients
Brown powder	500 mg	Caffeine
Codeiphen	250 mg	Acetylsalicylic acid codeine
Coffiplex	150 mg	Caffeine
Dolviran® (Bayer)	200 mg	Acetylsalicylic acid codeine caffeine barbiturate
Saridon® (Roche)	250 mg	Acetanilid caffeine
Dialyfen	500 mg	Barbiturate
Phenacetin	500 mg	
Coffeo-phenacetin	500 mg	Caffeine
Phenacetyl	250 mg	Acetylsalicylic acid
Diemalnatrium cum phenacetin	250 mg	Barbiturate
Codazin	250 mg	Codeine
Capazetyl	150 mg	Acetylsalicylic acid caffeine
Coffazin	250 mg	Caffeine
Pastor Fredrik s powder	500 mg	
Caca tablets	—	Acetylsalicylic acid caffeine

feature (4, 11 13, 14) In some cases of renal papillary necrosis haemolytic anaemia has been found (12)

Bengtsson (2 a) stated that there is strong evidence that renal papillary necrosis is a morphologic variant of chronic nonobstructive pyelonephritis Many writers stated that phenacetin per se is insufficient to cause papillary necrosis, and that infectious involvement of the tubules must supervene (8 10, 17)

The clinical picture in true chronic pyelonephritis consists of episodes of cystopyelitis, pyuria and bacteriuria usually with impaired renal function and slowly progressing uraemia Renal papillary necrosis whether associated with abuse of analgesics, diabetes or obstructive nephropathy, presents two main clinical patterns (1) An acute septic condition with rapid renal failure (16), and a subacute form with recurrent renal colic and haematuria when papillary fragments are sloughed into the urine

Own investigations

The records of all the cases of *chronic non obstructive pyelonephritis* treated at the Medical Clinic of Landspitalinn Reykjavik from 1957 through 1963 were studied Special note was made of the patients habits concerning use of analgesics Abuse was considered to have existed when at least one gram of phenacetin or compound analgesic was ingested daily for at least three years The minimum consumption thus exceeded one kg

The following criteria were employed for diagnosis of chronic nonobstructive pyelonephritis Pyuria bacteriuria impairment of renal function (hyposthenuria and/or hyperazotaemia) characteristic histological (biopsy necropsy) and roentgenological findings and a history of one or more attacks of cystopyelitis

Patients with diabetes mellitus collagenosis myelomatosis tumour acquired or congenital

malformations of the urinary tract and primary renal calculus were excluded from the series even if the criteria were otherwise fulfilled

The series comprised 43 female patients and 6 males i e 87 7 per cent females — cf Bengtsson s (2 a) series in which 93 per cent were females The age range was 17 to 80 years

The main analgesic preparations available in Iceland during the period covered by the study are shown in table I Many of them can be purchased without a doctor s prescription (Unless otherwise stated these preparations were manufactured under the control of the Icelandic Pharmaceutical Organization)

Of the 49 cases 19 (*group B*) had a history of long standing abuse of analgesics according to the stated criterion This is a somewhat higher frequency than the 25 to 30 per cent in comparable published series (14 22) The ages of the 19 patients (13 women and 6 men) ranged from 40 to 70 years which approximates to figures reported by other writers It is noteworthy that in 2 of the 19 cases (both men) there was habituation to tablets containing acetylsalicylic acid and caffeine but not as far as could be elicited phenacetin

In the 30 cases of chronic nonobstructive pyelonephritis with no history of analgesic abuse (*group A*) the age range was 17 to 80 years in 22 of them it was 40 to 70 years All the patients were females Absence of males among such cases has previously been reported (2 b

The distribution of the cases according to age and history concerning analgesics is shown in fig 1

All of the 19 patients who had been habituated to analgesics had pyuria and disturbed renal function with hyposthenuria and/or hyperazotaemia All showed specific renal lesions on roentgenological examination and/or characteristic histological changes in the kidneys In 11 cases there was no recorded history of cystopyelitis or bacteriuria Similar findings have earlier been presented (5) Six of the 19 patients had consumed analgesics

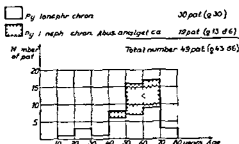


Fig 1 Age distribution of patients with chronic nonobstructive pyelonephritis during the period 1957 through 1963

for many years before bacteriuria appeared Bengtsson (2 a) stated a somewhat higher figure in this last respect

In all of the 30 cases without habituation to analgesics the records showed at least one episode of cystopyelitis Pyuria bacteriuria and hyposthenuria or isosthenuria were found in all cases and hyperazotaemia in 14 Histological examination was performed in 2 cases and showed typical chronic pyelonephritis

Roentgenological examination was performed usually more than once in all but 2 of the 49 patients during the period 1957—1963 The two women who were not roentgenologically examined had clinically unmistakable chronic pyelonephritis In 8 cases only plain roentgenograms were taken or urograms were unsatisfactory one of the reasons being uraemia The roentgenological findings are summarized in tables II and III Since 3 patients had undergone nephrectomy for hydronephrosis due to stone the total number of kidneys in the 47 roentgenologically examined patients was 91

At follow up examinations the roentgenologist was told only that the diagnosis was chronic pyelonephritis based on the mentioned criteria The course treatment and history with respect to analgesic consumption thus were not stated In 45 of the 47 examined patients one or more roentgenological signs of pyelonephritis were found Only 2 urograms showed no abnormality

In 13 cases the roentgenograms showed characteristic renal papillary necrosis ac

TABLE II Roentgenological findings in cases clinically diagnosed as chronic pyelonephritis

	No of kidneys	No of patients
Total	91	47
Calculus	16	10
Dilatation and scarring	36	27
Hypoplasia—shrunken kidney	5	5
Kidney size measurable	77	
Kidney size diminished at follow up (bilateral diminution in 3 cases)	23	

TABLE III Frequency of renal papillary necrosis and analgesic abuse in 47 roentgenologically examined patients

	Analgesic abuse	No analgesic abuse
Clinical diagnosis		
Chronic pyelonephritis	18	29
Roentgenological findings		
Renal papillary necrosis	11	2
Chronic pyelonephritis	6	26
Normal	1	1

cording to Lindvall s criteria (10) All of the 6 males in the series had renal papillary necrosis and in 2 of them the diagnosis was histologically confirmed *post mortem* Eleven of these 13 patients had a history of abuse of analgesics

At the end of the observation period, 5 of the 19 analgesic habituated patients (*group B*) were dead of uraemia Four of the 30 patients with no history of analgesic abuse (*group A*) were dead of uraemia at the time of follow up The others were re examined or were questioned as to their current state of health

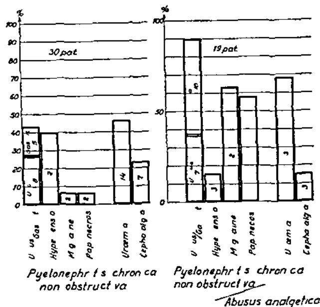
Influence of analgesic abuse on the clinical picture

In fig 2 the symptoms in the 19 analgesic habituated patients (*group B*) are compared with those in the 30 patients whose records did not show excessive intake of analgesics (*group A*) The symptom pattern in the former group differed considerably from the usual clinical picture in chronic nonobstructive pyelonephritis

Migraine In 12 of the 19 cases in *group B*, the records described migraine over periods ranging from 15 to 40 years and in 3 cases there had been persistent headache of other types Of the 30 patients comprising *group A*, 2 had a history of migraine and 7 of other varieties of headache The difference between the two groups as regards incidence of migraine is statistically significant ($P > 0\ 01$) Headache was the usual reason for heavy use of analgesics

Gastric disorder In *group B*, 7 patients had peptic ulcer and 10 had gastritis The corresponding figures in *group A* were 8 and 5 Here, too, the difference between the groups is statistically significant ($P > 0\ 01$) The gastric symptoms in *group B* appeared 4 to 12 years after analgesic abuse started (fig 3) Other writers have reported occasional cases with similar features (17, 18)

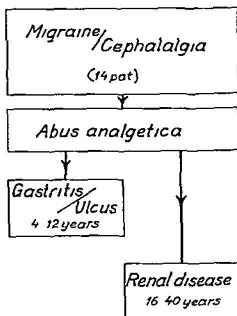
Renal papillary necrosis The frequency of roentgenologically demonstrated renal papillary necrosis in *group B* was 58 per cent (11 of 19 cases) In 2 of these 11



F g 2 Ma n symptoms and s gns n the case series

cases of renal papillary necrosis careful questioning and study of all available records revealed prolonged abuse of compound acetylsalicylic acid and caffeine analgesics but not of phenacetin Only 2 patients in *group A* presented roentgenological signs of renal papillary necrosis The intergroup difference is highly significant (P > 0 001)

Hypertension The blood pressure was elevated in 3 of the 19 patients in *group B* a frequency resembling that in the general population (6) Similar findings were previously published (5 20) Hypertension was present in 12 of the 30 patients in *group A* which corresponds to the frequency reported by Bengtsson (2 a) The groups thus did not differ statistically with respect to incidence of arterial hypertension



F g 3 C n cal course of the disease n pat ents hab tua ed to phenacet n

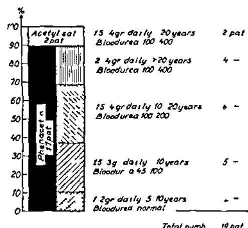


Fig 4 Relationship between blood urea and consumption of phenacetin containing analgesics

Uraemia This complication occurred in 13 cases from *group B* and in 14 from *group A* The difference is not statistically significant

Haemolytic anaemia was detected in 3 cases from *group B*, but in none from *group A*

The clinical data are summarized in figs 2 and 3

Case reports

The following case histories were typical of chronic nonobstructive pyelonephritis associated with analgesic abuse

Case 1 A 61 year old woman had a history of migraine since the age of 35 years During the following 20 years she took 3 to 10 brown powders (each containing 500 mg phenacetin) daily After 5 years of this medication a gastric ulcer was diagnosed Ten years later at the age of 50 she had a severe attack of renal colic with haematuria Urography showed no stone but the urine was described as thready After another 5 years she switched from brown powders to Hydergin® (Sandoz) with favourable effect on headaches and general health Renewed recourse to the powders 2 years later (1959) was followed after some months by another attack of renal colic with haematuria Tissue fragments were passed in the urine but urography revealed no stone Anaemia uraemia and chronic pyelonephritis were diagnosed at this hospital in the following year and renal papillary necrosis in 1963 During the past 3 years her renal status has remained fairly stable The blood pressure is 140/80 mm Hg She ceased to take large doses of analgesics in 1960

Case 2 A 60 year old man started to take brown powders (8 to 10 daily) at the age of 38 years because of headache — suspected migraine Gastritis was diagnosed 4 years later His history of kidney trouble dated from 1954 13 years after phenacetin abuse began Seven years after the initial renal symptoms he had an attack of renal colic but neither stone nor infection was found Isosthenuria and hyperazotaemia were discovered in the following year (1962) The urinary sediment contained leukocytes erythrocytes and bacteria but cultures of the urine were negative The haemoglobin value was 86 per cent (13 2 g %) and the blood pressure 190/120 mm Hg Urography revealed bilateral shrunken kidney with papillary necrosis

Comments

In 14 of the 19 cases in which pyelonephritis was associated with protracted abuse of analgesics, there was a triad of gastritis or peptic ulcer, renal disease and cephalalgia The gastric disorder was diagnosed 4 to 12 years and the renal lesions 16 to 40 years after analgesics abuse began Fig 3 illustrates the course of the disease in these typical cases Renal papillary necrosis occurred in 11 of the 14 cases

It has not been demonstrated that phenacetin alone irritates the gastric

ucosa, but most of the ingested drugs ontained also acetylsalicylic acid caf ine, etc The irritant and ulcer roducing action of these substances on e gastric mucosa is well known (3) Moreover, large doses of acetylsalicylic cid can cause proteinuria and haema ıria (3)

Fig 4 illustrates the relationship be ween blood urea levels and consump on of analgesics The blood urea clearly ncreased with the intensity and duration f analgesic abuse

Conclusions

n this series of hospital in patients with hronic nonobstructive pyelonephritis he pattern of symptoms in the patients who had been habituated to analgesics differed distinctly from that in the group with no known analgesic abuse

Habituation to analgesics was induced n most cases by persistent headache usually migraine Gastritis or peptic ulcer developed as a rule after some years and renal papillary necrosis was commonly found in this group of patients Statistical analysis showed the frequency of these findings to be signif icantly greater than among the patients whose pyelonephritis was not associated with heavy use of analgesics

The blood pressure was normal in most of the cases of analgesic habituation

Chronic nonobstructive pyelonephritis is rare in men and it may seem remark able that in all of the 6 male cases in this series there had been protracted abuse of analgesics

Summary

During the seven year period 1957—1963 49 cases of *chronic nonobstructive pyelonephritis* were studied at the De partment of Medicine at Landspitalinn, Reykjavik The diagnostic criteria were pyuria, bacteriuria, impaired renal function, characteristic roentgenological and histological findings and one or more attacks of cystopyelitis

Patients with diabetes mellitus collagenosis, myelomatosis tumours acquired or congenital genitourinary malformations and "primary" renal calculus were excluded from the series

Of the 49 patients comprising the series, 19 had consumed excessive amounts of analgesics over long periods The analgesics contained phenacetin in 17 cases but as far as could be ascertain ed from careful investigation, only acetylsalicylic acid and caffeine in 2 cases The clinical and roentgenological manifestations in these 19 patients differed markedly from those in the 30 patients with no history of analgesic abuse

Habituation to analgesics was induced in most cases by persistent headache usually migraine As a rule these patients developed symptoms of gastritis or peptic ulcer after some years of heavy analgesic consumption In 11 of the 18 roentgeno logically examined cases in this group including the 2 with habituation to acetylsalicylic acid caffeine analgesic renal papillary necrosis was found The incidence of headache gastric disorder and renal papillary necrosis was significantly higher than among the cases without excessive consumption of anal-

gesics Most of the patients in the analgesic habituated group were normotensive

The series contained 6 male patients All 6 had a long history of analgesic abuse and all had roentgenologically verified renal papillary necrosis

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Results of Instrumental Transventricular Commissurotomy

A Follow up Study

By

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Surgical treatment of mitral stenosis is generally regarded as useful Even a moderate widening of the stenosed mitral ostium may help to improve the patient s symptoms Previously employed digital rupture was soon found to be an insufficient method in fibrous scarred and sclerotic valves In later years several instrumental methods have been developed (29) One of these methods has during the last years been employed at the Thoracic clinic of Sahlgrenska sjukhuset

In order to assess the results of this operative technique a follow up study was carried out on the patients operated during the period 1960—1962

Material and methods

The series included 82 patients 26 males aged between 27 and 64 years with a mean age of 49 years and 56 females aged between 27 and 67 years with a mean age of 47 years Fifteen patients had previously undergone digital commissurotomy

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In most of the patients heart catheterization was carried out in addition to the clinical evaluation Pre operatively the patients were classified according to the criteria of the New York Heart Association

The follow up examination took place 6—40 months after the operation The mean time of observation for the entire series with elimination of the patients who had died during the first post-operative month was 20 months All the patients alive at the follow up study were clinically examined and in all except three ECG and chest roentgenograms were taken The patients were re-classified according to the same criteria as before operation

The effects of surgical intervention on clinical condition and physical working capacity were evaluated The following four classes were used to describe the results

The first class included improved patients who had attained a better physical condition and thus shifted to a better function group

The second class consisted of subjectively ameliorated patients who had not improved their physical working capacity and thus still remained in the pre-operative functional group

The third class comprised patients with further deterioration of functional capacity following operation

The fourth class included the fatal cases

Results

In the first class there were 29 females and 10 males, comprising 47 per cent of the total material More than half of the women belonged to this class

Sixteen females and ten males, altogether 32 per cent of the total material were included in the second class There were many reasons for the lack of increase of their physical working capacity Several of these were not directly related to the technical results of commissurotomy Five patients, for example could not increase their physical working capacity due to hemipareses, four patients due to rheumatic reactivation or post-commissurotomy syndrome and one due to myocardial infarction during the convalescence

The clinical condition became worse in one female and two males altogether 4 per cent of the total material, who were thus included in the third class

There were 14 deaths recorded 10 females and 4 males altogether 17 per cent of the total number of patients Six out of these patients died during the operation due to rupture of the atrium, ventricle or one of the valves. Four patients died during the first month following the operation. Three of these four had physical signs of mitral insufficiency and died with symptoms of increasing cardiac failure. The fourth patient developed a wound infection with subsequent rupture of the sternotomy sutures and finally died from cardiac and pulmonary insufficiency Five of the ten patients who died during the operation or during the first post-operative month underwent the valvulotomy because of re-stenosis

The remaining four deaths out of 14 occurred 2, 12, 18 and 35 months respectively, after the operation The respective causes of death were circulatory failure due to pneumonia, myocardial infarction, mitral insufficiency and aortic stenosis

It is reasonable to assume that the results of operation were affected by several factors Many of these can be assessed pre-operatively, others not until the actual operation A compilation of some important factors has been made in figs 1—6

Fig 1 shows *the age* of the patients in relation to the post-operative classes 62 per cent (50 patients) were aged between 41 and 56 years In the majority of the cases (30 of 39) re-classified into a superior function group the age was 51 years or less, while the age of the ameliorated patients who were somewhat improved but not re-classified (17 of 26) exceeded 51 years However, a male aged 64 years has improved and has been re-classified. The age distribution among the dead patients on a whole is in accordance with that of the entire series The oldest patient in this series was a 67 year-old female who died from myocardial infarction one year after the operation

Fig 2 shows the relation of *pre-operative functional groups* according to the New York Heart Association criteria to the post-operative classes More than half of the series (50 of 82 patients) belonged to Group II before the operation and only three to the poorest functional Group IV The others had been classified in Group III More than half of the improved patients who had

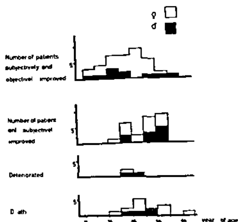


Fig 1 Operation results and age distribution (26 males 56 females)

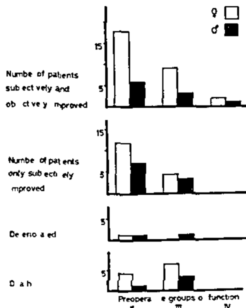


Fig 2 Pre-post operative classification according to the New York Heart Association Scheme

been re classified (24 of 39) pre operatively belonged to Group II thus after the operation they were classified in Group I Twelve of the 29 patients in the pre operative Group III and the three in Group IV improved and were re classified Eight of these have been moved even two groups upwards (5 from Group III and 3 from Group IV) A considerable improvement has thus resulted in these seriously ill persons Most of the patients who became somewhat better but were not re classified post-operatively (19 of 26) belonged to Group II The others were classified in Group III Of those whose condition had become worse two were in Group II prior to operation and one in Group III Of those who had died during the operation three belonged to Group II and three to Group III Of the remaining eight who died two were in Group II and six in Group III

Fig 3 relates patients with pre-operative *mitral insufficiency* of a slight degree to post operative classes The diagnosis

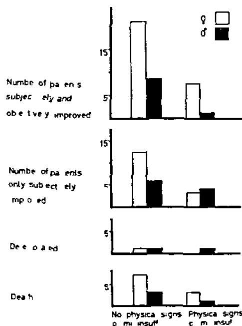


Fig 3 Operation results in relation to pre operative slight mitral insufficiency No increased operation risk

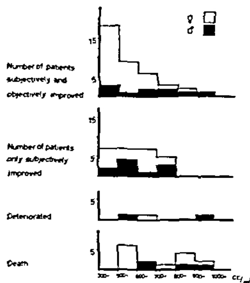


Fig 4 Operation results in relation to pre operative heart volume.

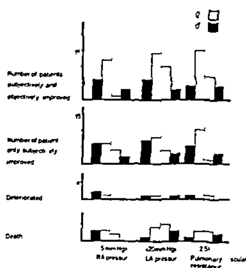


Fig 5 Operation results in relation to pre operative pressure at rest

is based on the apical, harsh, pansys tolic murmur of degree 3 or more About 1/4 of the patients (21 of 82) had that type of systolic murmur After the operation nearly 50 per cent of the patients (9 of 21) revealing the above mentioned findings on auscultation had become improved and re-classified The slight pre-operative mitral insufficiency did not jeopardize the results of opera tion in this series

Fig 4 shows the *pre-operative heart size* expressed in ml/sqm body surface area (BSA) *in relation to post-operative* classes The patients have been classified into six groups the first with a volume of less than 500 ml, the second with a volume of 5—600 ml etc All 26 of the patients who had a heart volume of less than 500 ml became improved and most of them (19 of 26) were also classified into another group Three of the patients remained in the same group post operatively because complications such as hemiparesis and rheumatic reactiva tion and/or post-commissurotomy syn drome prevented an improvement of their capacity Out of the patients with a heart volume of 500—800 ml/ sqm, 18 were re-classified, 17 not objec tively improved, two deteriorated and nine died Out of the patients with a heart volume of 800 ml or more, three were objectively improved, one deterio rated and five died Out of the patients who died during the operation, four had a heart volume less than 800 ml and two above

Fig 5 shows the *pre operative pressure at rest* in the right and left atria and the pulmonary vascular resistance in relation to the four post-operative classes More than half (20 of 33) of the improved, re-classified patients had a pressure below 20 mm in the left atrium Too much importance should not be attached to the pre-operative pressure and flow measurements at rest as a guide for the

assessment of the immediate operative risk or the post-operative results

Pre operatively 32 of 82 patients had *sinus rhythm* and the remainder had atrial fibrillation All the cases with sinus rhythm improved except one, and out of these, all except seven had improved objectively Three of these seven cases revealed reasons clearly explaining the lack of an improved capacity post operatively, viz hemiparesis or post commissurotomy syndrome Out of the 50 patients with atrial fibrillation, about 1/3 (15 pat) were re classified in a superior group and approximately 1/3 (19 pat) remained unchanged Two became impaired and the remaining (14 pat) died

In order to reduce the risk of *thrombo embolism* during operation the patients with atrial fibrillation were generally treated with anticoagulants for at least three weeks prior to operation In four of these patients who survived during the first post operative month, a thrombosis was found in the left atrium at the operation, but in no case were there any emboli during the time of observation Out of thirteen patients with atrial fibrillation who had not been treated with anticoagulants or who had received this treatment for less than three weeks five cases had thrombosis of the atrium, two of whom developed emboli

Fig 6 shows the frequency of *calcification* in the valves, observed at the operation in relation to post operative classification Calcification was more common in males than in females Three fourths of the objectively improved patients (27 of 37) revealed no calcification About half of the improved

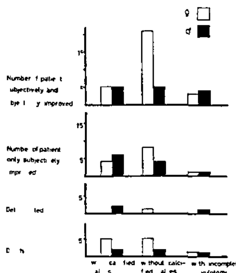


Fig 6 Operation results in relation to changes in the mitral valves

number of patients who had not been re classified (10 of 22) had calcification, the others not Among the patients in whom calcification had been observed the mortality was higher (7 of 29) as compared to the rest (7 of 47) The result of the operation furthermore was less successful Only in one case did calcification cause an embolus A male was afflicted with persisting hemiparesis

Fig 6 further shows the frequency of *incomplete ruptures* In twelve cases the surgeon only managed to cause a rupture in one of the commissures Despite this, seven of these patients could be re classified in better function groups

The number of patients with mitral insufficiency assessed by the auscultatory findings was pre-operatively 21 Post-operatively the corresponding figure was 36 Out of the latter 19 had been re classified to a superior function group three had deteriorated and three died

TABLE I Literature on method and results of operations (percentage)

Author and year	Method	No of patients	Results[1] Excellent/good
American College of Chest	—	5 482	75
Physicians 1959		342	70
Werko 1959	—	—	25–66/30–40
Glenn & Stewart, 1961	—	—	45–50/25–30
Baker & Hancock 1959	—	239	85
Kunos et al 1961	—	1 000	58/25
Taber & Lam 1961	Closed	226	65
		200	42
Lowther & Turner 1962	—	200	57
Gialloreta & Tardif 1964	—	389	27/50
Ellis et al 1959	Digital	1 000	46/23
			36/19
Mason 1960	Digital	435	60/25
Baden 1958	Digital (112) instrumental (17)	129	33/34
Irmér et al 1960	Digital or instrumental	1 000	41/39
Engell & Fabricius 1961	Digital (102) instrumental (14)	116	62
Keith et al 1963	Digital (90) instrumental (4)	94	32/24
Belcher & Gupta 1964	Digital (8) instrumental (trans ventr) (92)	100	21/27
Logan et al 1962	Instrumental (transventr)	84	56
Own material	Instrumental (transventr)	82	47/32
De Jesus et al 1962	Closed (39) Open (4)	43	51
Jaderberg & Nilsson 1964	Digital (47) instrumental (transventr) (7) Open (2)	56	20/39

[1] Some authors state the percentage of the survivors others that of the entire series

No attempt to evaluate quantitatively the degree of insufficiency has been made

There were 15 patients who were re operated because of *re stenosis* Six were re classified, three only subjectively im proved and five died during the opera tion or during the first post operative month and, furthermore, one patient died 35 months after the operation The mortality rate during operation or during the first post operative month among these patients was thus higher than in the remainder (33 and 7 per cent respectively)

The roentgenograms of the heart taken at the follow up showed that in three patients the heart volume had increased by more than 100 ml/sqm BSA and that in six patients it had

	Mortality			
Fair/poor	At oper	Late	Obs time (year)	Remarks
19/6	1 5—1 4		1/2	Report based upon 10 000 operated patients
26/4			5	
ca 15/8—15	5—15			List of literature up to 1959
15—20/1—5	2—6			List of literature up to 1961
15	11 3			Follow up of 200
13	3 6	3 9	1—8	100 of them were followed up within a year
25	3 5	6 5	2—8	Sinus
30	15	13	2—8	Atrial fibrillation
36	7		6	
22	7 5	15 1	5—12	262 followed up
9/13	3 1	8	2—9	Group III (705) followed up
11/15	23 6	19	2—9	Group IV (206) followed up
15	9 5		Not stated	1st series 1950—55 400 followed up
11/6	9	7	1—5	
20	6 5		Not stated	300 followed up
	4 3	11	1—5	77 followed up
14	9	14	<5	34 patients
			>5	32 patients
				Of whom 5 were re operated
21/13	5	12	1—6	Re-operations* 94 followed up
32/6	2 4	6	1—8	Re-operations 50 followed up
4	12	5	1/2—3 1/2	
19	23	7	Not stated	Re-operations
25	9	7	1—9 5	Pre-operatively
				6 Group II
				33 Group III
				17 Group IV

* Re operation only when stated otherwise first operation

decreased by more than 100 ml Out of the three whose heart volume had increased, two deteriorated and one improved and was re classified Out of the six whose volume had decreased, four were re classified and two only subjectively improved In the latter cases one had hemiparesis and the other reactivated rheumatic inflammation

Eight patients who improved considerably after operation and who were re classified in two superior functional groups were younger than 50 years On the other hand only four of them had sinus rhythm and only two of these had a heart volume less than 500 ml/sqm BSA prior to the operation Re stenosis of the mitral valve was the cause of the present operation in three of these eight patients

Three patients who deteriorated after operation developed severe pansystolic murmur highly suggestive of mitral incompetence One of these three patients had an aortic valvular lesion of slight degree, a heart volume exceeding 1,200 ml/sqm BSA and pulmonary changes of restrictive type

Discussion

An extensive literature exists on the medical and surgical problems of mitral stenosis (11, 17, 26, 35, 37) as well as reports concerning the results of surgical treatment (2, 3, 9, 10, 12, 13, 14, 16, 19, 25, 31, 34) Several papers have been published on the results of transventricular instrumental commissurotomy (1, 6, 8, 15, 27, 29, 32)

It is difficult to compare the results of surgical treatment between different series of patients Several factors influence the results such as the selection of patients, technique of operation, the surgeon s experience and the time of follow up In table I we have attempted, however to summarize the results of different types of operation extracted from selected publications In several reports the assessment of the results had been made without provision of an accurate statement of the patients functional capacity No direct and definite conclusions can be drawn from the results presented in this table, but certain tendencies are obvious in the different series In the present series an excellent improvement was obtained in 47 per cent and good in 32 per cent Post-operative deterioration was noted in only 4 per cent of the total material These results seem to be in a rather good accord with the majority of the results presented in table I Due to the comparatively short time of observation (6—40 months) the results could not be markedly affected by re stenosis or heart failure due to a traumatic mitral regurgitation

The mortality rate varies considerably in different series (table I and II) In this series the mortality rate in immediate connection with the operation and during the first post operative month was 12 per cent In the groups of patients where operation was performed because of re stenosis, the mortality rate was much greater both with the closed and the open techniques and figures up to 35 per cent have been stated (24, 36) In the present series of patients the mortality rate of 12 per cent during the operation and the first post operative month could be thus divided into the mortality in connection with the first operation which was 7 per cent and that in connection with the reoperation which was 33 per cent

Factors influencing the results of surgical treatment have been discussed by many authors Some of the most important factors which have been discussed in the literature are presented in table III Generally, the factors causing an increased risk at the operation influence also the results in a negative direction concerning short term as well as long term prognosis The incidence of re stenosis is greater among the patients who constitute a poor risk at the first operation As favourable and unfavourable factors usually occur in the same patient, it is difficult to deter

TABLE II Literature on method and mortality rate of operation

Author and year	Method	No of patients	Mortality rate of operation (%)	Remarks
Harken et al 1961	Digital	80	9	Re-operation[1]
Ellis & Harken 1964	Digital	1 215	2 7	Group III
		356	22	Group IV
	Digital or instrumental (transventr)	139	5 8	Group III Re-operation
			17 4	Group IV Re-operation
Dogliotti et al 1958	Digital or instrumental	2 000	3 1	Mortality rate of last 500 pat 1 4%
Carlgren et al 1959	Digital or instrumental	100	2 0	
Logan & Turner 1959	Digital	388	5 0	32 of them were re-operated
	instrumental (transventr)	438	6 4	(12 5% mortality rate)
Gerbode 1960	Digital or instrumental (transventr)	200	12 5	17 of them were re-operated (12 5% mortality rate)
Irmer et al 1960	Digital or instrumental	1 000	6 5	
Mason 1960	Digital (35) instrumental (transventr) (165)	200	2 5	2nd series 1958—59
Bjork et al 1961	Digital (60) instrumental (90)	150	9 0	In 22 pat simultaneous dilatation of aortic orifice
Crum & Tsapogas 1959	Instrumental (transventr)	50	6	5 of them were re-operated All deaths were poor risk cases
Austen & Wooler 1960	Instrumental (transventr)	100	3	8 of them were re-operated
Linder 1961	Instrumental (transventr)	35	0	9 of them were re-operated
Dubost et al 1962	Digital or	965	2	
	instrumental (transatr)	38	8	Re-operation
Lowther & Turner 1962	Instrumental (transventr)	92	2 4	Re-operation
Own material	Instrumental	67	7	
	(transventr)	15	33	Re-operation
Kay & Zimmerman 1962	Closed	200	4	This has been reduced to 5% in
	Open	106	8	the last 40 patients
Taber & Lam 1963	Closed	39	25	Re-operation
	Open	45	35 5	Re-operation
Bittencourt et al 1962	Open	89	10	
Junqueira et al 1962	Open	40	12 5	
Nichols et al 1962	Open	141	6 0	
		29	13 8	Re-operation

[1] Re-operation only when stated otherwise first operation

TABLE III Influence of various factors on operation results (percentage)

Author and year	No of patients	Sex		Cardiothoracic ratio (%)		Age	
		♂	♀	<60	>60	Younger	Older
Ellis et al 1959	1 000	83/72	85/71			[1]91/80	[2]73/63
		68/50	71/57			[1]81/67	[2]11/56
Lowther & Turner	500			4	[2]14	5	[2]18
1962	500				20/48		10/32
	185			64	50	63	41
Gialloreta & Tardif 1963	262						
Belcher & Gupta 1964	100						16/25
Ellis & Harken 1964	1,571						

[1] Age group 20—29
[2] The authors state that the difference is significant

mine to what extent each separate factor has affected the result of the opera tion In many patients factors other than those mentioned in table III may have been of considerable importance for the prognosis

Some of the factors influencing the outcome of the operation in the present series of patients are discussed below

The age seems to affect the results of operation in the present series as well as those presented in table III The best results were achieved in patients younger than 51 years of age in our series Good results were, however, obtained also in patients older than 60 years Relatively high age should thus not necessarily be regarded as an absolute indication against operation (17, 30, 37)

The degree of *pre operative functional incapacity* as expressed in terms of func tional group according to the New York Heart Association criteria is of the greatest importance with respect not only to the risk at operation (table IV) but also to the later prognosis (tables I and III, Ellis et al) In the present series the best results were obtained in patients belonging to functional Group II Baden (2) compared the results of surgical and medical treatment in pa tients classified in functional Groups II—IV and found no considerable difference with regard to the mortality rate between the two groups during the observation period of two years With respect to functional capacity, however, the majority of surgically treated patients were in a better condition than the patients treated medically Ellis et al (13) found that up to nine years of observation, the survival rate among those operated upon in functional Groups II and III was 71 per cent In a compar

Rhythm		Calcifications		Mitral insuff		Obs time (year)	Remarks
Sinus	Auricular fibrillation	Not heavy	Heavy	Not heavy	Heavy		
88/83	80/69			88/77	75/50	1	% improved group II – III/IV
78/65	60/51			78/69	*48/36	5	
2	*13	4	*16				% mortality rate of operation
	37/84		16/48				% survivors/died at operation
69	46	63	52			6	% improved
	11/38		24/52			5–12	% excellent improvement/poor
	60/80		35/45			1–6	% good improvement/poor Only re-operation
1 8/15	3 8/24						% mortality rate of operation Group III/IV

* Age group 50—59 Otherwise border between younger and older 50 years

TABLE IV Mortality rate of operation (percentage) in various function groups

Author and year	No of patients	Group I and II	Group III	Group IV
Baden 1958	129	–	10 0	24 0
Irmer et al 1960	1 000	0 5	5 3	22 6
Mason 1960	200	1 7	2 2	14 0
Ellis & Harken 1964	1 571	–	2 7	22 0
Gialloreta & Tardif 1964	389	5 4	7 6	10 6

able Danish series receiving medical treatment the survival rate was approximately 40 per cent

In group IV the survival rate in the Ellis et al series after nine years was 57 per cent (43 per cent dead 24 per cent at operation) while no medically treated patients (Danish series) survived after eight years In the present series, the three patients belonging to Group IV improved markedly In view of the fact that surgical therapy improves the prognosis considerably as compared to medical operation seems to be indicated in the selected patients belonging to the *functional* Groups II III and IV (11, 17, 37)

Moderate or severe *pre operative mitral insufficiency* affects the operative result (table III) According to the report from the American College of Chest Physicians (34) the mortality rate of operations in 'Pure mitral stenosis' was 6 3 per cent in Predominant

Mitral Stenosis 8 per cent, and in Combined mitral stenosis' 14 per cent In the present series the mortality rate was not higher in cases of slight mitral insufficiency The three patients in this series whose condition deteriorated post-operatively all developed traumatic mitral insufficiency at the operation In many cases of combined mitral valvular lesion it is difficult to determine the degree of mitral insufficiency Contrast injection in the left ventricle during angiocardiography may be of value trying to quantitate the amount of regurgitation in order to facilitate surgical correction according to the open technique (11, 17, 36)

The size of the heart may have an effect on the risk of operation as well as on the post-operative result (table III) A cardiothoracic ratio of less and more than 60 per cent respectively carried a mortality rate of 4 and 14 per cent respectively There was a good result after six years in 64 and 50 per cent respectively according to Lowther and Turner (30) In our series the best results were found in patients with a heart volume less than 500 ml/sqm BSA Baden (2) observed that there were no good results in patients with very large hearts (cardiothoracic ratio exceeding 65 per cent) *However three patients in the present series with a heart volume* of more than 800 ml/sqm improved considerably while eight patients with cardiac size 500—700 ml/sqm died Pronounced heart enlargement may give a poor result in a larger number of patients but is no definite indication against operation (37) The cause of the heart enlargement should be carefully analysed and adequate pre-operative treatment should be instituted before a definite decision concerning surgical intervention is made (37)

Baden found no connection between the *pre-operative pulmonary vascular resistance* and the result of the operation He found that the pressure in the pulmonary artery was of greater importance than the pulmonary wedge pressure His patients in Groups III and IV who before the operation had a pressure in the pulmonary artery exceeding 50 mm Hg at rest, responded with more pronounced haemodynamic improvement than others In the present series no relation was found between the pre operative catheterization results obtained at rest and results of the operation The results of post operative recatheterization show that the pressure become normalized more frequently by the use of an open technique as compared to the closed (33) Better results are obtained also by means of the instrumental method than of digital (32)

The rhythm of the heart and the functional condition of the patient are important factors from the point of view of prognosis Table I (35) and table III show that atrial fibrillation increases the risk of operation and makes the long and *short term prognoses worse In the present material atrial fibrillation was* present in all patients who died and in two of the three whose condition deteriorated post-operatively

The frequency of pre-operative *thrombo-embolism* is about 13 per cent among the patients submitted to operation (7 10, 34) In Carlgren's series (7) the percentage of emboli in patients with

moderate (< 600 ml) and those with a more severe (> 600 ml) heart enlargement was about the same In atrial fibrillation, thromboses in the atrium are found at the operation at a rate of up to 20 per cent (10) In 15 patients with atrial fibrillation who had not been treated with dicumarol, Carlgren et al (7) found five cases of intra cardial thromboses and in 35 patients treated with dicumarol the same number was noted In Carlgren's series embolism occurred in 6 per cent at operation and according to the report from the American College of Chest Physicians (34) in 5 3 per cent, 2 8 per cent of which were fatal In a partial report of the latter compilation, the frequency of arterial operative embolism in atrial fibrillation was 8 3 per cent and in sinus rhythm 2 5 per cent Dubost et al (10), who treated all cases of atrial fibrillation with anticoagulants during the last month prior to operation, had in 1009 patients only nine with arterial emboli, six of whom were fatal About half of the present patients were operated upon during continued treatment with dicumarol which had been started at least three weeks earlier Zachrisson et al (38) compared this group with the untreated one and found that among those treated there was no increased tendency to bleeding and probably less tendency to thrombo embolism Atrial fibrillation is thus not an indication against operation Patients with this rhythm disturbance should, however be pre treated with anticoagulants (17)

Calcification of the valves is more frequent in higher ages and more common in men than in women (17, 30) The latter observation also applies to this series This difference between men and women is believed to be one of the causes of the poorer operative results in men (12) Heavy calcification of the valves make them more rigid and immobile Commissures can be caused to rupture only with difficulty Despite a successful rupture the mobility of the valves is only slightly improved with a poor functional result as a consequence (table III) Also in this series the result was poorer in patients with calcified valves Patients with marked calcification of the valves should be operated upon with the open technique in order to prevent incomplete rupture, traumatic mitral insufficiency and emboli (36)

Incomplete commissurotomy is a frequent cause of lack of improvement of the patient This should not be confused with re-stenosis which may occur after successful surgery and may be due to reactivation of the rheumatic infection The longer the patient survives after the operation the greater the risk is for *re stenosis* Lowther and Turner (30) reported an increased frequency of re stenosis from 5 per cent five years after operation to no less than 70 per cent after nine years' follow up in a comparatively small number of patients The shortest interval between the first operation and the operation for re-stenosis was only one year (4)

Experiences of 100 operations for re stenosis show that the results of the second operation were not as satisfactory as after the first but that nearly half of the patients still improved considerably (4) If the suspicion of re stenosis

arises the patient should be subjected to a new investigation including if possible, cardiac catheterization and angiocardiography with injection of contrast in the left atrium, the left ventricle or both Surgery according to the open technique should be considered (36)

Lowther and Turner analysed the factors influencing the results of operation in a series of 500 patients Two groups were selected one consisting of the patients who had only one of the three so-called adverse factors, viz atrial fibrillation, heavy calcification of the valves and prominent cardiac enlargement, while the second group included all patients who did not have any of these adverse factors The mortality rate was higher among the patients who had atrial fibrillation as compared with the second group and also with all patients in sinus rhythm The higher mortality rate was thought to be due to higher frequency of thromboembolism in the patient group with atrial fibrillation Heavy calcification of the valves and prominent cardiac enlargement respectively were not accompanied by increased mortality rate as compared with the second group These two features taken together had a definite negative influence as was evident from comparison with cases having no more than one of these features This suggests that one of the three adverse factors may not itself be of decisive importance but may play an important role together with certain other signs of impaired myocardial function The mobility of the valves and the condition of the myocardium should thus be taken into account when surgery is under discussion Angiocardiography can be of help in evaluation of the mobility of the valves, but assessment of the condition of the myocardium is more difficult Cardiac enlargement and atrial fibrillation may be due to mechanical consequences of the valvular deformation and/or to myocardial insufficiency (30) In a group of 23 patients with cardiac enlargement, atrial fibrillation and chronic or recurrent congestive failure, 5 patients died in connection with the operation due to cardiac arrest, circulatory failure or thrombo embolism (30)

The most marked improvement after operation is not uncommonly noted in patients belonging to the pre operative functional Group IV It is impossible to know whether purely mechanical factors, alone or in conjunction with myocardial damage, were the cause of the poor pre operative condition Measurement of the end diastolic pressure in the left ventricle under a standardized load might prove valuable for determining the condition of the myocardium

According to the available literature, indications for open surgery seem thus to be mitral regurgitation, thrombo embolic masses in the left atrium and heavy calcification of the mitral valves Concomitant lesions in the aortic valves and/or the tricuspid valves constitute a further reason for open surgery according to Taber and Lam (36) Mortality rates in the present series argue for open surgery against closed in practically all cases with the possible exception of patients with sinus rhythm or cardiac size less than 500 ml/sqm BSA

Furthermore, functional results could be expected to be better after the open valvulotomy than after the closed as judged from the frequency of disappearance of mitral murmurs (33) It ought to be remembered however, that when two different surgical methods are compared with each other, possible differences in the patient material as well as the experience of the surgeons with these methods must be taken into account The need for a well planned study which fulfils the basic statistical requirements seems to be undisputable

Summary

The series includes 26 males and 56 females, 15 of whom had re stenosis On an average the time of observation was 20 months The patients were classified pre and post-operatively according to the principles of the New York Heart Association

After the operation 47 per cent of the patients could be classified into a superior function group, 32 per cent had improved subjectively, but were not re classified 4 per cent had become worse and 17 per cent died Among the latter there were also four deaths (5 per cent) which occurred after the first post operative month The mortality rate in connection with the first operation was 7 per cent, and at the operation of re stenosis, 33 per cent

All the patients with a heart volume less than 500 ml/sqm body surface area and with a sinus rhythm improved and most of them could be re classified into a superior group after the operation The mortality rate had not increased among the patients whose age exceeded 51 years, nor among those belonging to Groups III and IV The slight pre operative mitral insufficiency, based merely on the apical pansystolic murmur, did not increase the risk of operation Heart catheterization and pressure re cording was of no great importance for assessing the risk of operation or the prognosis In one case there was a complication of embolus caused by calcification Patients with atrial fi brillation were treated with anticoagu lants for at least three weeks prior to the operation These patients escaped thrombo embolic complications

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Fever of More than Two Weeks' Duration

By

H Fransen and L E Bottiger

Fever of long duration and unknown origin is commonly encountered by the diagnostician but rather few analyses of clinical series are recorded in the literature Most papers report follow up investigations of patients discharged with a "diagnosis" of fever of unknown origin (2, 4, 9, 10, 12) Some authors examined patients admitted to hospital for fever of unknown origin (5) or hospitalized patients who had fever of long duration (11) Geraci (8) emphasized the importance of laparotomy to diagnosis The results of some of these studies are given in table I As will be seen there infections predominated although their frequency varied widely in different series (25 to 65 per cent), neoplastic diseases were of fairly constant incidence (16 to 30 per cent) collagen disorders would appear to be increasing in frequency (0→ 20 per cent) These results reflect the panorama of diseases in different countries at different times, but they are not directly comparable since the criteria of selection and the definition of fever varied

For practical purpose it seemed of interest to analyse an unselected hospital series comprising all cases of fever of unknown cause The present paper reports a 12 month series from Stockholm s Infectious Diseases Hospital owing to its special conditions of admission, this hospital serves a large proportion of the patients with fever of unknown origin in the Stockholm area

Material

The series studied comprised all the patients who had *fever for more than two weeks* while hospitalized at Stockholm s Infectious Diseases Hospital during 1960 Fever was defined as a morning temperature of *more than 37 4 C* for several consecutive days and *over 38 0 C* on at least one occasion In every instance *a final fall in temperature* showed that fever had in truth been present Body temperature was measured rectally

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TABLE I Results in different authors series (disease distribution expressed in per cent)

Author	Hamman Wainwright	Keefer	Bottiger	Geraci et al	Petersdorf Beeson	Petterson
Year	1936	1939	1953	1959	1961	1962
Number of cases	54	75	34	70	100	25
Infections	59	65	46	24	36	60
Tuberculosis	17	11	17	7	11	36
Pyogenic	20	49	20	13	22	24
Others	22	5	9	4	3	0
Neoplastic diseases	22	20	30	30	19	16
Carcinoma	15	13	24	16	11	12
Lymphoma	7	7	6	14	8	4
Collagen diseases	0	11	12	8	15	20
Miscellaneous disorders	0	4	12	24	23	4
No diagnosis	19	[1]0	[1]0	14	7	[1]0

[1] Not included in the investigation Taken in part from Petersdorf & Beeson (11)

The case records revealed that a confident diagnosis was arrived at after two weeks fever in altogether 287 (83 per cent) of the 347 cases which came into the category studied (tables II and IV) In another 33 cases a diagnosis was obtained before discharge The remaining 27 patients — listed as FUO in table V — were followed up during 1964

The age and sex distribution of the series will be found in table III As is seen there women predominated (64 per cent) and the majority of patients were over the age of 50 years No distinct difference emerged in these respects between the cases in which a diagnosis was arrived at and those in which none was established However the group in which the diagnosis was unknown after two weeks contained more women than the known diagnosis group ($0.05 < P < 0.01$)

Results

A Diagnosis known after two weeks fever

The results are given in table IV *Infections* predominated over half of them involving the *lungs and upper respiratory tract* The age distribution of the patients with pneumonia was studied especially, and it emerged that a very large proportion of the patients over 50 years (125 = 57 per cent) had pneumonia If these cases are excluded from the highest age groups, the incidence of fever shows only a slight rise with advancing age

Other infectious diseases included a wide selection of different conditions, foremost among which were infections of the central nervous system and gastrointestinal tract, and septicaemia Many of the patients with *tuberculosis* and fever were transferred early — that is, before two weeks had elapsed — to specializing hospitals so that this group is not fully comparable with the others

The *tumour group* was dominated by leukaemia mainly chronic lymphatic

TABLE II Patients with fever for more than 2 weeks

Diagnosis established within 2 weeks		287 (82 7 %)
Diagnosis not known after 2 weeks but made before discharge		33 (9 5 %)
Diagnosis unknown at time of discharge		27 (7 8 %)
Total number of patients with fever for more than 2 weeks		347 (100 %)
Total number of admissions	5 182	
Proportion of fever cases		6 6 %

leukaemia, and by carcinoma of the lung Of the other conditions, cirrhosis of the liver with 17 per cent fever cases merits special mention

B Diagnosis unknown after two weeks fever

Table V gives the final diagnosis in the 60 cases in which it was not arrived at until the patient had had fever for more than two weeks The diagnosis was in every instance in which it was established made during the period of hospitalization in which the fever occurred The follow up investigation afforded a probable diagnosis in only two instances, leaving 25 cases labelled as fever of unknown origin

In this group of late diagnosis, *tuberculosis* predominated among the *infectious diseases* — being finally diagnosed in five cases In none of these instances was tuberculosis suspected initially, the chief symptom in them all was high fever of long duration, for weeks to months Data on these five cases are given in table VI More detailed reports on two of them follow below

Case 2 (*table VI*) Housewife 47 years who had during her youth been treated for pleurisy Since 1958 she had received corticosteroids for a diagnosis of lupus erythematosus disseminatus Admitted to hospital for a routine check Suffered from arthralgia Repeated roentgen examination of the lungs showed nothing definitely abnormal During the first few weeks the temperature was subfebrile subsequently

TABLE III Age and sex distribution

	Diagnosis after 2 weeks fever			
	Established	Unknown	Total	
Age				
< 50 years	66	14	80	36%
> 50 years	221	46	267	64%
Total	287	60	347	100%
Sex				
Men	111	15	126	23%
Women	176	45	221	77%
Total	287	60	347	100%

TABLE IV Diagnosis known within two weeks of fever

	Total admissions	Fever of 2 weeks duration	Fever cases of total admissions (%)
Infectious diseases			
A Lungs and upper respiratory tract			
Bronchopneumonia pleuropneumonia	414	89	22
Influenza + pneumonia	184	43	23
Bronchitis	118	10	8
Primary atypical pneumonia	31	5	16
Other infections of upper respiratory tract		8 155	
B Others			
Meningitis encephalitis	120	17	15
Cystitis cystopyelitis	145	16	11
Gastroenteritis	295	10	3
Septicaemia (including endocarditis)	—	8	
Mononucleosis	122	7	6
Hepatitis (infectious)	146	5	3
Erysipelas scarlet fever with complications		4	
Tuberculosis	55	3	5
Herpes zoster	16	2 72	13
Neoplastic diseases			
Leukaemia	10	5	50
Carcinoma of lung	17	5	29
Others		2 12	
Other diseases			
Cirrhosis of liver	52	9	17
Rheumatic diseases		7	
Colitis	55	5	9
Diverticulitis of colon		4	
Allergic diseases		3	
Cholecystitis		1	
Temporal arteritis	7	1	
Sarcoidosis	7	1	
Diverse diseases (often several concurrent causes of fever)		17 48	
Total		287	

successively rising and fluctuating Two months after admission an abscess developed at the left wrist and histologic examination showed it to be a granulomatous process in which acid fast bacteria were demonstrable Another roentgen examination of the lungs revealed small radio-opaque patches throughout the lung fields One week after the start of adequate therapy the patient was afebrile She is in good health but the mobility of the large joints is appreciably restricted and lung function is reduced

Case 3 (table VI) Male, 23 years, Finn Admitted to a Military Hospital for fever two years earlier The present onset was marked by intermittent abdominal pain and fever of 38° C Referred to hospital as a suspected case of cholangitis with high fever The erythrocyte sedimentation rate rose, the transaminases were slightly elevated, and the Mantoux test was strongly positive As the abdominal pain subsided and nasal samples showed growth of *Staphylococcus aureus*, penicillin was administered This had no effect on the body temperature Ledermycin® subsequently led to afebrility and the patient was discharged in good condition Three weeks later he again developed high fever and abdominal pain On admission the erythrocyte sedimentation rate was 80 mm/hour and the urine diastase was 1 512 A suspicion of pancreatic irritation prompted laparotomy, which disclosed a fist sized tumour at the site of the pancreas, oedema of the surrounding tissues and numerous glands along the aorta Biopsy tuberculosis of the lymph glands

Neoplastic diseases predominated in the group of patients with fever of late diagnosis The prognosis was poor only one of the 19 patients survived four years later The largest group was composed of patients with carcinoma of the hepatic, biliary, or pancreatic region, most of them over 60 years, all subfebrile and referred to hospital for jaundice investigation Explorative laparotomy was diagnostic in two cases, biopsy in two, while autopsy afforded the diagnosis in two cases Owing to the presence of jaundice diagnosis was directed to the liver and biliary tract — but diagnosis nevertheless took some time In four of our six cases the erythrocyte sedimentation rate was fairly low, 10 to 30 mm/hour

TABLE V Final diagnosis in fever cases in which it was unknown after 2 weeks fever

	No of cases	
A. Infectious diseases		
Tuberculosis	5	
Staphylococcal septicaemia	1	
Mononucleosis	1	7
B Neoplastic diseases		
Carcinoma of pancreas liver or gall bladder	6	
Carcinoma of lung	5	
Hodgkin s disease	3	
Carcinoma of colon or stomach	2	
Carcinoma of ovary	1	
Carcinoma of kidney	1	
Reticular cell sarcoma	1	19
C Other diseases		
Sarcoidosis	2	
Temporal arteritis	2	
Allergic diseases	1	
Ulcerative colitis	1	
Cholecystitis with abscess	1	7
D Fever of unknown origin		27
Total		60

Carcinoma of the lung seems strikingly often to give rise to fever Of the altogether 17 cases seen during the year five were in the group in which a diagnosis was arrived at before two weeks had elapsed and a further five in the late diagnosis group Thus fever was present in some 60 per cent of these cases Most of the patients, all of whom were over 50 years, were from the start believed to have bronchopneumonia but roentgen examination revealed progressive lung lesions and the high erythrocyte sedimentation rate of 50 to 100 mm persisted The three patients with Hodgkin s disease all had high, fluctuating fever without periodicity

TABLE VI Clinical and laboratory data on five patients with tuberculosis

Case	Sex	Age	*Preliminary diagnosis*	Clinical data	Mantoux test
1	♂	30	Hodgkin s disease	High fever cough headache	—
2	♀	47	Lupus erythematosus disseminatus	Arthralgia subfebrile later high fluctuating fever, articular abscess	?
3	♂	23	Recent duodenal ulcer abdominal observation	Abdominal pain and fever for 2 months	+
4	♂	64	Bronchopneumonia observation for paratyphus	Fever for 1 month	—
5	♂	50	Pleurisy	Fever for 2 weeks vomiting antibiotics at home	—

sternal puncture, liver biopsy, and laparotomy, respectively were diagnostic

Under the heading of *other diseases* there were two cases each of sarcoidosis and temporal arteritis diagnosed by biopsy

remaining 16 were in good health, afebrile and with a normal erythrocyte sedimentation rate, and no explanation for their usually lengthy period of fever had been found during the four to five years since their hospitalization

Follow up

The results are given in table VII Nine patients had died, most of them within 12 months of discharge from hospital In most instances the cause of death was such that the same disease may have given rise to the fever on the earlier occasion of hospitalization

Among the 18 survivors, a probable diagnosis was arrived at in only two cases (a woman with a renal carbuncle and a man with rheumatoid arthritis) The

Discussion

For several reasons our results differ from those reported by other authors As was mentioned in the introduction, the criteria of selection varied in different series We included patients with only a slight elevation of body temperature although this was invariably shown by a later fall to have been true fever If as often applies only patients *discharged* with a diagnosis of fever of unknown origin are included

Lab data	Chest X rays	Final diagnosis
ESR 2—40 mm SGOT 49 SGPT 74	On admission nothing abnormal 3 weeks later miliary lesions	Miliary tuberculosis
ESR 80—91 mm Acid fast bacteria in abscess	On admission suspect infiltrates 3 months later miliary lesions	Miliary tuberculosis and lupus erythematosus disseminatus
ESR 57—80 mm SGOT 78 SGPT 122	Nothing abnormal	Tuberculosis of retroperitoneal lymph glands
ESR 92—100 mm	Infiltrates , later increasingly suggestive of tuberculosis	Tuberculosis of right lung
ESR 60—91—65 mm *SGOT* 53 SGPT 122 Acid fast bacteria in pleural effusion	' Pleurisy	Tuberculous exudative pleurisy

TABLE VII Follow up of patients discharged with a diagnosis of fever of unknown origin

	Sex	Age	No of cases	
Alive				
Probable fever cause				
Renal carbuncle	♀	25	1	
Rheumatoid arthritis	♂	72	1	
No demonstrable fever cause	♀	33—82	16	18
Dead				
Heart disease	♀	62—77	5	
Pneumonia	♀	77	1	
Necrotic jejunal ulcerations	♀	80	1	
Gynecological neoplasm	♀	81	1	
Post operative death (2 years after fever period)	♂	79	1	9
Total				27

those who succumb during hospitalization are ruled out as are the cases of fever of long duration in which the correct diagnosis is established before discharge The character of the hospital and its situation also affect the results The Infectious Diseases Hospital in Stockholm admits a very large propor

tion of all the patients who develop fever of unknown origin — as also, of course, those with fever of known infectious causation

During the nineteen thirties, rheumatic fever, septicaemia, and syphilis predominated as causes of fever in series of fever of uncertain cause Rheumatic fever has almost entirely disappeared, syphilis has become far more uncommon, and attention is instead directed chiefly to malignant tumours and systemic diseases

Tuberculosis has been a conspicuous factor in all studies on fever cases, recently most striking in Petterson's investigation from Finland (12) Our findings confirm the view earlier expressed by Böttiger et al (6), that tuberculosis is an important source of fever of unknown origin, and that a negative chest roentgenogram should not exclude tuberculosis from consideration It is often extrapulmonary tuberculosis which escapes diagnosis Two of our five patients had miliary tuberculosis, an articular abscess being diagnostic in one of them, one had tuberculosis of the abdominal lymph glands one pleurisy and only one had solely a parenchymatous lesion in the lung

At an infectious diseases hospital like that in Stockholm, naturally infections are initially sought as causes of fever, and it follows that most of them are rapidly detected Accordingly, the group in which diagnosis was late included only one case of septicaemia in which diagnosis was delayed by partly contradictory results of blood cultures

On the other hand, and not wholly unexpectedly, tumour cases predominated in this late diagnosis group The incidence of carcinoma of the lung was particularly high That diagnosis is not easy to make early, many patients have concurrent infection which impedes evaluation of the roentgen appearances and expectant therapy is often preferred — sometimes for too long (1) 'The first roentgenographic abnormalities due to lung cancer are highly variable and often unimpressive", as emphasized by Boucot et al (3) in an extensive prospective study of more than 6,000 men Time can almost certainly be saved if attention is given from the start to diagnosing or ruling out carcinoma of the lung, and not delaying this decision until other results are negative In suitable cases lung biopsy might perhaps contribute to better and earlier diagnosis Several simple instruments for taking lung biopsy specimens have been constructed

Another diagnosis which may often take time is carcinoma of the abdominal organs Here, too, early biopsy — especially of the liver — must surely improve diagnosis

The follow up study afforded a probable explanation for the earlier febrile period in only two cases As has been pointed out (5) the majority of the cases discharged from hospital as fever of unknown origin remain undiagnosed subsequently However, the signs and symptoms suggest that in several cases the disorder was rheumatic polymyalgia (Bagratuni s disease), a condition increasingly often diagnosed in older persons characterized by muscular pain and fever, a high erythrocyte sedimentation rate and anaemia (7)

Summary

The diagnosis for all patients who had fever for more than two weeks while hospitalized at Stockholm s Infectious Diseases Hospital during 1960 was analysed The 347 fever cases accounted for 6 6 per cent of the total number of admissions

The diagnosis was arrived at within two weeks in 287 cases (83 per cent) The majority of these patients (227 or 79 per cent) had infectious conditions

The diagnosis was not established until more than two weeks had elapsed in 33 cases (9 per cent) In this group the neoplastic diseases predominated (19 cases = 58 per cent), particularly carcinoma of the lung or abdominal organs

The diagnosis remained unknown in 27 cases (8 per cent) during hospitaliza tion, nor was it established with confi dence on follow up

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Moniliasis — an Internal Disease?

Three Cases of Idiopathic Hypoparathyroidism with Moniliasis, Steatorrhea, Primary Amenorrhea and Pernicious Anemia

By

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The first cases of idiopathic hypoparathyroidism were described by Beumer and Falkenheim (4) in 1926 In 1939, when 14 cases were published Drake et al (12) considered that the following criteria should be fulfilled to justify a diagnosis of idiopathic hypoparathyroidism a) low serum calcium b) high serum phosphorus, c) chronic tetany d) absence of roentgenological signs of rachitis and osteomalacia, e) absence of steatorrhea, renal insufficiency and alkalosis For the diagnosis of idiopathic hypoparathyroidism it is now a days also necessary to demand a satisfactory phosphorus diuresis after an intravenous injection of 200 units of parathyroid hormone (Ellsworth Howard test) In 1942, Albright et al (1) found signs of subnormal phosphorus diuresis after injection of parathyroid hormone in three patients who conformed to the conditions suggested by Drake et al (12) Albright et al (1) termed this new syndrome pseudo hypoparathyroidism (Seebright Bantam syndrome) In 1958, Bronsky et al (5) collected 50 cases of idiopathic hypoparathyroidism, which not only showed satisfactory phosphorus diuresis subsequent to injection of para thyroid hormone, but also conformed to the conditions of Drake et al (12) Eight of these 50 patients suffered from moniliasis, five from Addison's disease and one from pernicious anemia Seven of the cases were subjected to post mortem examination, and in six of them no parathyroid glands could be found The seventh case had normal parathyroid glands, but on microscopic examination it was found that the normal structure had been replaced by adipose tissue In four of the cases, the suprarenal glands were atrophic

Moniliasis or Addison s disease have not been observed in patients suffering from pseudo hypoparathyroidism (5) The few microscopic examinations of parathyroid glands available have shown normal or hyperplastic glandular tissues

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TABLE I Summary of reported cases with more than one of the following diseases hypoparathyroidism moniliasis, hypoadrenalism, pernicious anemia and steatorrhea

Author		Hypoparathyroidism	Moniliasis	Hypoadrenalism	Pernicious anemia	Steatorrhea	
1 Thorpe et al	1929	+	+	−	−	−	
2 Rowntree et al	1931			+	+		Autopsy
3 Sevringhaus	1942	+	+	−	−	(+)	Gastro-intestinal instability
4 Talbot et al	1943	+	+	+	−	−	
5 Sutpin et al	1943	+	+	−	−	−	Sibling of case 6 and 7
6 Sutpin et al	1943	+	+	−	−	−	
7 Sutpin et al	1943	+	+ ?	+ ?	−	−	Tuberculosis of the suprarenes?
8 Sutpin et al	1943	+	+	−	−	−	
9 Sutpin et al	1943	+	+	−	−	−	
10 Leonard	1946	+	−	+	−	−	Autopsy
11 Gotta et al	1948	+	+	−	−	−	
12 Collins Williams	1950	+	+	−	−	+	Sibling of case 20
13 Berlin	1952	−	−	+	+	−	
14 Salvesen et al	1953	+	+	−	−	+	
15 Leifer et al.	1953	+	(+)	+	−	+	Candida parapsilosis
16 Papadatos et al	1954	+	−	+	−	−	
17 Massachusetts General Hosp	1954	+	+	−	−	−	Case 40361 Autopsy
18 McLean	1954	+	−	−	−	−	Ptos bilat
19 Craig et al	1955	−	+	+	−	−	Autopsy
20 Craig et al	1955	−	+	+	−	+	Autopsy
21 Craig et al	1955	+	+	+	−	−	Autopsy
22 Reisner et al	1955	+	−	−	+	−	
23 Perlmutter et al.	1956	−	−	−	−	−	Autopsy
24 Whitaker et al	1956	+	+	+	−	(−)	Autopsy Diarrhea
25 Hurwitz	1956	+	−	−	+	−	
26 Forbes case 1	1956	+	−	+	−	−	
27 Forbes case 3	1956	+	−	−	−	−	Autopsy
28 Forbes case 7	1956	−	+	−		−	
29—31 O Donovan	1957	−	+	−	−	−	
32 O Donovan	1957	−	+	−	−	+	
33 Walsh	1957	+	−	−	−	−	Case A O M
34 Wilkins	1957	+	−	−	−	−	
35 Di George et al	1957	+	−	−	−	−	Sibling of case 36
36 Di George et al	1957	+	+	−	−	−	
37 McMahon et al	1959	+	−	+	(+)	+	Autopsy
38 Williams et al.	1959	+	−	(+)	−		Autopsy
39—40 Cramblett	1959	−	+	+	−		
41 Wagner	1960	+ ?	+	−	−	−	
42 Wagner	1960	−	+	+	−	−	Autopsy

Table I Cont

Author		Hypoparathyroidism	Moniliasis	Hypoadrenalism	Pernicious anemia	Steatorrhea	
43 Clarkson et al	1960	+	−	−	−	+	
44 Buzdygan et al	1961	−	+	−	+	+	Hypothyroidism
45 Buydygan et al.	1961	−	+	+	−	−	Autopsy
46 Morse et al	1961	+	+	−	+	−	Sibling of case 47
47 Morse et al	1961	+	−	+	(+)	+	Mucoviscoidosis?
48 Morse et al	1961	+	−	+	−	+	Case N L.
49 Halmos et al	1962	+	−	−	(+)	+	No megaloblast cells
50 Hung et al	1963	+	+	+	+	−	Two siblings Addison s disease
51 Kunin et al.	1963	+	−	+	+	+	
52 Ikkala et al.	1964	+	−	−	+	−	
53 Kenny et al	1964	+	+	+	−	+	Hashimoto s thyr
54 Kenny et al	1964	+	+	−	−	−	Sibling of case 52
55 Quinto et al	1964	+	+	+	+	−	
56 Hiekkala	1964	+	+	+	−	−	
57 Hiekkala	1964	+	+	−	−	−	Sibling of case 56
58 Hiekkala	1964	+	+	+	−	−	Sibling of case 56
59 Hiekkala	1964	+	+	+	−	−	Sibling of case 56
60 Sjoberg	1965	+	+	−	−	+	Sibling of case 61
61 Sjoberg	1965	+	+	−	−	−	Amenorrhea prim
62 Sjoberg	1965	+	+	−	+	−	

(1, 16) and thus bears out the theory of renal insensitivity to parathyroid hormone resulting in low phosphorus diuresis in these patients

During last ten years, approximately ten cases have been described in which the patient, in addition to idiopathic hypoparathyroidism and possibly also moniliasis, simultaneously has suffered from steatorrhea or pernicious anemia Earlier steatorrhea was thought to have no connection with idiopathic hypoparathyroidism (12) but Jackson (24) considers idiopathic hypoparathyroidism and steatorrhea to be a new syndrome These patients have a high serum phosphorus, not a low value such as would exist in the event of the patient suffering from typical sprue Table I enumerates the cases which have shown symptoms of more than one of the abovementioned diseases (idiopathic hypoparathyroidism, moniliasis Addison s disease pernicious anemia steatorrhea) The three last mentioned cases (cases I—III in Case reports) are all new, and refer to idiopathic hypoparathyroidism and moniliasis Case I has also had transient pancreatitis and steatorrhea, case II primary amenorrhea and case

5 7 mg per 100 ml She was obstipated and complained of paresthesia in her legs She got a rapid reduction of hemoglobin to 6 8 g per 100 ml Physical examination revealed a pronounced glossitis incipient cataract but no signs of papilledema

Laboratory data Hemoglobin 6 8 g per 100 ml Index 1 30 She had a histamine fast achylia Serum iron 135 μg per 100 ml B_{12} in serum 0 004 μg per 100 ml Folic acid normal No haptoglobins present There were numerous megaloblasts in the bone marrow Diff count revealed nucleated red cells 22/200 white X ray examination now showed calcifications in the basal ganglia No signs of resorption defect, and function tests showed normal conditions Oral glucose-tolerance curve, however, rather flat 17 ketosteroids in urine normal Growth of Candida albicans in pharynx and on nails Antibody titer against Candida albicans 1/120 The patient was treated with vitamin B_1 injections, with a reticulocyte response *of 33 per cent and a rise in hemoglobin to* 12 g per 100 ml In addition, the patient is taking 15—20 drops of A T 10

The patient s parents and a younger brother are perfectly healthy and show normal values for calcium phosphorus, hemoglobin and red corpuscles and have no signs of moniliasis

Comments

The described three cases are unquestionably examples of idiopathic hypoparathyroidism Case I has had temporary steatorrhea, caused partly by disturbances in the function of the small intestine and partly by pancreatitis Subsequent to the treatment with A T 10 the steatorrhea disappeared The same observations have been made by several other authors (7, 11, 26, 30 33) Clarkson et al (7) are of the opinion that the patient s hypoparathyroidism with hypocalcemia could be the cause of the intestinal symptoms Salvesen and Böe (43) established the existence of Candida albicans in feces from a patient suffering from steatorrhea Williams and Wood (53), on the other hand, discussed the possibility of a cause common to the hypoparathyroidism and the steatorrhea

Before the correct diagnosis was established cases II and III were treated as epilepsy for a considerable time Subsequent to the first appearance of the disease with epileptic seizures, both cases have shown normal values of serum calcium between the attacks In case III, the patient suffered attacks in direct connection with estrogenic treatment for primary amenorrhea Ranney (40) proved an antagonism between estrogenic and parathyroid gland hormones Estrogenic hormone tends to increase the accretion of calcium As often happens, the diagnosis of case III was established in connection with morbilli This patient also had a temporary ptosis as described in at least two other cases in the literature (30 33) All three patients showed changes in tooth enamel, but roots of the teeth were normal This may possibly indicate that the disease developed comparatively late in life, or had been very mild prior to the age of 10—12 years The enamel changes in case II, however, have most likely occurred during the earliest years of life

Patients suffering from idiopathic hypoparathyroidism show a varying state of the teeth but as a general rule the teeth are very bad when the disease has developed early Both enamel and

dentine are hypoplastic and sometimes certain teeth may entirely be lacking (2, 29) Development of the disease in later years has no effect on the teeth, as holds also for patients with post-operative hypoparathyroidism (29)

In 1955, pernicious anemia was first observed in patients suffering from idiopathic hypoparathyroidism Since then, 8—10 additional cases have been reported, several of them referring to children This is remarkable, in view of the fact that pernicious anemia is unusual in children and adults under the age of 30 In one case of idiopathic hypoparathyroidism with steatorrhea (7), the B_{12} serum values showed a temporary reduction and in some cases gastric hydrochloric acid reappeared when serum calcium values reverted to normal (19, 23) Case III had already in 1963 at the age of 22 hyperchromic anemia (index 1 13) but her hemoglobin was 11 5 g per 100 ml The peripheral blood contained nucleated red cell as well as Howell Jolly corpuscles When the disease became manifest in the autumn of 1964, the nucleated red blood cells changed to 22/200 white cells This has no connection with the common form of pernicious anemia and has previously been observed in one case of megaloblastic anemia + idiopathic hypoparathyroidism (26) In all other respects, the patient suffers from quite a typical megaloblastic anemia, and has also reacted favorably to B_{12} treatment The dermatological changes in case III during 1963 thought to be indicative of the Steven Johnson s syndrome may well have been caused by moniliasis The changes in mouth, vagina and anus were very similar to those seen in cases of Candida albicans

Discussion

What part does Candida albicans play? Ever since Sutphin et al (45) in 1943 published their five cases with idiopathic hypoparathyroidism and moniliasis, many investigators have discussed the significance of moniliasis The majority seems to be of the opinion that Candida albicans is an innocent secondary finding Against this, however, it may be remarked

1) First fungus appearance, often long before symptoms of tetany
2) Moniliasis does not develop in cases of pseudo hypoparathyroidism (5) or post operative hypoparathyroidism (11, 35, 45, 53)
3) Fungus infections do not heal with treatment of the patient s idiopathic hypoparathyroidism
4) Exceptionally high antibody titers against Candida albicans in the three cases presented

In recent years a revaluation of Candida albicans has taken place subsequent to reports of several serious conditions caused by moniliasis Any organ appears to become the seat of the mycosis (54) Previously described cases of pernicious anemia and pancreatitis simultaneously with moniliasis may then be better understood It may not be possible, however, to explain Addison s disease and idiopathic hypoparathyroidism in this way since Candida albicans has never been observed on autopsy in parathyroid and suprarenal glands (5,

26, table I) Perhaps, however, Candida albicans might produce toxins which are released into the blood stream and act on these endocrine organs, causing atrophy to occur. A form of allergy may also be suspected, in view of the fact that Candida albicans, as for Trichophytons, can produce powerful so-called "id" reactions, i.e. the skin becomes sensitized for the fungus. Candida albicans has not been traced in these "id" reactions. In patients suffering from idiopathic hypoparathyroidism and Addison's disease, the parathyroid glands and suprarenal glands would thus have been made sensitive to the action of the fungus or its toxins in a similar manner. Several authors have wondered whether an auto-immune reaction is the actual cause of the disease. In many cases antibodies against suprarenal glands, the stomach mucous membrane and even against the thyroid gland have been found in the blood. It is, of course, possible that these antibodies constitute merely a secondary phenomenon and arise only when cellular proteins from the respective endocrine organs have entered the blood stream subsequent to the endocrine cells having been destroyed by toxins or allergy.

Titers for antibodies against Candida albicans have been made in a few cases. The value of such titer tests is difficult to assess in view of the fact that antibodies against Candida albicans may be found in 13—64 per cent of the population according to various investigators (13, 14, 34, 37, 48). Of patients with clinical moniliasis 75 per cent showed a positive complement binding reaction (37). However, only 3 per cent of the material investigated by Todd (48) showed a titer higher than 1/150. Beemer et al. (3) suggested that high titers may have diagnostic significance. All three of our patients had high titres from 1/120 to 1/480. It would appear unlikely that this should be a coincidence.

Summary

Three cases of idiopathic hypoparathyroidism and moniliasis are described. All three cases show long standing changes of Candida albicans on their nails, and high antibody titers against Candida albicans. Case I has had an acute pancreatitis and signs of malabsorption, and during the period of observation case III developed megaloblastic anemia with B_{12} deficiency. A survey is furnished in respect to cases with more than one of the following diseases: idiopathic hypoparathyroidism, moniliasis, Addison's disease, pernicious anemia and steatorrhea. In the discussion it is asked whether moniliasis might play a central role in the pathogenesis of the different syndromes. The protean and confusing character of the idiopathic hypoparathyroidism may be explained in this way. For the diagnosis and treatment of idiopathic hypoparathyroidism more attention should be directed to the possibility of an infection with Candida albicans.

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Quantitative Bacterial Culture of Urine

I A Preliminary Report on a Method and on the Limit between Contamination and Significant Bacteriuria

By

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Bacteriuria, first described by Roberts (21) in 1881, has been accepted as an expression of infection in both the upper and the lower urinary tracts By definition, bacteriuria signifies multiplication of bacteria in the urinary tract and consequent growth of bacteria on culture of the urine under optimal conditions, the condition being present either alone or accompanied by pyuria

However, this diagnosis has the drawback that bacteriuria might also result from contamination with habitual organisms on the external genitals Many investigators (3 5, 6 7, 15 20, 22) have demonstrated that in normal men and women the urethra harbours bacteria Thus, positive culture is in itself not enough to permit the conclusion that urinary tract infection is present Passage of a catheter, whereby the bacterial flora of the urethra is bypassed, does not exclude the possibility of contamination (5 6)

The inadequacy of the criteria in common use i e positive results of culture and of microscopical examination for urinary infection gradually have become evident It was therefore necessary to find other techniques and criteria for the clinical evaluation of urinary tract infection

Marple (16) in 1942 solved the problem by introducing into clinical work the principle, long established in bacteriology, of quantitative culture of bacteria from urine

The method, however, did not come into common use until Kass (11, 12) in 1955 revived it and ensured its wide acceptance by his publications Considerable reservation with regard to quantitative urine culture was expressed from many sides At the present however, it seems that this principle of quantitative urine culture has found its place in the diagnostic armamentarium

The discussion on quantitative urine culture centers on the problems of finding easy and reliable methods and of establishing a definite boundary between contamination bacteriuria and

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Fig 1 "Mid-stream-urine-set" consisting of test tube with hydrophobic cotton wool tampons The set is packed in "steril" bags and dry sterilized

significant bacteruria, and thus elucidating the real nature of bacteriuria its aetiology pathogenesis and not least its epidemiology and ecology

The aim of this study is to give a preliminary report on a bacterial counting technique and on a method of urine collection both of which have proved to be well suited to routine investigations being found rapid economical and reliable and to be usable outside bacteriological laboratories The study further aims at establishing the boundary between contamination and significant bacteriuria as evaluated on the basis of a normal material satisfying a variety of criteria for healthy kidneys A survey of the methods of making bacterial counts and of the objections against them has been given elsewhere 29

Methods

The collection of urine was by means of the following midstream technique The mid stream set" shown in fig 1 was used this is a practical and hygienic disposable unit simpler than those used in the past.

In men the prepuce is retracted and the glans rinsed thoroughly with the three cotton wool swabs soaked in a disinfectant, which is poured into the aluminium bowl There upon the patient voids urine After half emptying the bladder the subjects directs the urinary stream into the sterile test tube which is filled half way and stoppered with the sterile cotton wool stopper

In women, the vulva and introitus are washed with sterile cotton wool swabs The last of these is left in the vagina to hinder contamination from possible vaginal discharge The patient begins to void urine and approximately half way through micturition a sterile aluminium bowl held by the patient is introduced into the stream of urine The urine is then decanted into the sterile test tube

Immediately after collection the sample is placed at approximately + 4 C.

The method has been checked against other methods of collection and has shown good agreement with catheter urine as has also been found by other investigators (18 Urine voided and collected in the usual manner gave considerably higher counts even if the subject had washed first Separate estimations of the hourly variation in the bacterial count in the same patient showed the time of collection to be of no consequence 28

The necessity of sterilizing the equipment for rinsing and collection has been demonstrated by other authors 13

Quantitative urine culture A "flood plate" modification was used 23 The examination was divided into two steps performed at an interval of 12—24 hours first a semi quantitative estimation and then a dilution test if warranted

1st step the urine was mixed thoroughly by repeated suction into the pipette and an aliquot of 0.5 ml was taken and inoculated on to a blood agar plate dried in advance The inoculum was distributed on the plate by rocking and the plate was then allowed to dry It was then closed and placed in an incubator at 35—37 C.

The plate was examined after 12—24 hours If less than 500 colonies were found the incubation was continued and counting performed after 36—48 hours but if more than 500 colonies were found the second step was performed

2nd step The requisite degree of dilution was estimated according to the growth on the plate thus with heavy growth five successive logarithmic dilutions were made and the dilutions 10^{-4} and 10^{-5} were used for inoculation With confluent but grainy growth, four logarithmic dilutions were performed and the dilutions 10^{-3} and 10^{-4} used for inoculation etc

The dilution 1 10 was prepared from 4 5 ml of sterile isotonic sodium chloride solution and 0 5 ml of stirred up undiluted urine which had been kept at + 4 C throughout For further dilutions 0 5 ml of this mixture was taken with a clean pipette etc For inoculation use was made of dried blood agar plates which in the case of Proteus species had been pretreated with 96 % ethyl alcohol for 3 min without the lid and thereupon dried for 15 min at 37 C The plates were finally incubated at 35 C—37 C for 36—48 hours

After the incubation the plates were counted with clear marking of the colonies For the sake of accuracy plates with colony counts below 30 or above 500 were discarded (30) For the same reason three plates should be incubated (25 31)

The average of the count was multiplied by 2 corrected for the dilutions used and the result given as colonies/ml urine

Precision of the method In separate experiments on the uncertainty of the method a standard deviation of 10 5 was found In calculations of the standard error of the mean the coefficient of variation was found to be 3 % which shows remarkably exactness These were separate experiments (28) however and in later routine assays (26 27) a somewhat greater coefficient of variation was found (10—15 %)

The results for repeated single tests were not influenced by an intervening large scale routine application of the technique

TABLE I Author s method compared with different semi quantitative methods

Method	No of specimens	Colonies/ml urine $<10^5$	Colonies/ml urine $>10^5$
Griess reaction pos	10	0	10
Griess reaction neg	252	239	13
> 5 leucocytes	21	9	12
< 5 leucocytes	332	307	25
Bacteria	31	0	31
No bacteria	324	316	8

Samples were collected daily for 13 days at the same hour from the same patient These samples were immediately cultured quantitatively The agreement of the results demonstrated the good reproducibility of the method (28)

The main difference between the technique described and that formerly used is the cooling to + 4 C separate assays (28) showed this cooling to be without significance for the first five days which is in agreement with the results of Mou and Feldman (19)

It may be taken for granted that any one culture consists of biologically different elements with different powers of multiplication different metabolism etc For a differentiated determination of the amount of organism any vital count including the one described here is inherently inadequate because of neglect of an elementary criterion — the morphology of the organisms The author s method has therefore been compared with direct microscopic counting and the results have been found to agree (28)

In further attempts to compare the different methods — the present method the end-point dilution method and the streakplate method (28) — the latter two were not found inferior to the author s The limitations of this quantitative culture method are quite few as mentioned earlier (11 28)

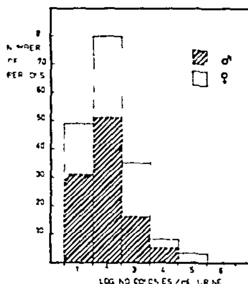


Fig 2 Incidence of bacteriuria in the normal series

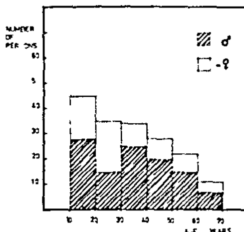


Fig 3 Sex age distribution in the normal series.

In addition a series of randomly selected midstream urine samples were examined in a comparison between the present method and some semi-quantitative methods used for estimating bacteriuria The results are given in table I It is seen that the Griess reaction involves a large percentage error, as also found by other authors (10 24)

It was found that the presence of more than 5 leucocytes per visual field is a poor diagnostic criterion in agreement with several other investigators

Material

During a given period all men and women without a history of kidney disease and who fulfilled the following conditions were selected from cases attending a casualty room 1 no present or past symptoms from the urinary tract 2 never any catheterization 3 no history of former surgical intervention involving the urinary tract or major gynaecological surgery 4 normal renal function as estimated from serum creatinine concentration (less than or equal to 1 2 mg/100 ml for both sexes (1) 5 no glycosuria, determined with Clinistix®, or proteinuria determined with Heller s test, 6 no arterial hypertension (maximum 160/110 mm Hg), 7 no disease or condition in which bacteriuria is considered to be a frequent complication (pregnancy, diabetes mellitus) 8 finally, those patients were excluded who were in poor general condition, as well as persons who had received chemotherapy or had been bed ridden within the last week

Patients with subjective symptoms of hypertrophy of the prostata or cysto-rectocele and obese persons (25 % above normal weight) were also excluded

Results

The sex and age distribution of the material is seen from figs 2 and 3

The average age of the women in the material was 31 7 years, and 36,2 years for the men

The distribution of the bacterial count/ml urine, and hence the incidence of bacteriuria among these 175 normal patients with anamnestical healthy kidneys, is seen from fig 3

Discussion

It appears from the literature that quantitative urine culture has become an established method of clinical diagno-

sis, but with the earlier methods there has been the handicap that the urine samples had to be diluted and inoculated within a rather short interval, so as to avoid further growth in the sample This is difficult to fit into routine work, and is timeconsuming and expensive if the number of organisms is completely unknown, since long dilution series are then necessary, demanding large amounts of medium and glass equipment

The author s experiments (28) have shown that it is possible to store urine containing bacteria at + 4° C for at least 5 days without significant alterations in the bacterial count This finding allowed the introduction of a semiquantitative estimation with a screening as it is possible to keep the urine in cold storage and to prepare the dilutions only in such cases and to such an extent as required The first step too could be used in the quantitative estimation

As mentioned earlier, Marple (16) was the first author to publish quantitative estimations of the bacterial content of the urine using a precise technique and performing the experiments personally, he was able to fix the limit at 10^5 organisms/ml urine Fourteen years later Sanford et al (23) described a method with modifications for routine clinical use, unfortunately at the cost of the precision of the method These authors establish the limit at 10^3 colonies/ml urine In a later discussion (12), they expressed the opinion however, that the limit is probably at a higher level, and in their subsequent publications (2 17) this higher limit is maintained as they operate with a limit of 10^5 colonies/ml urine

Studying a large group of unselected ambulant patients, Kass (12) found that the bacterial count in catheter urine from women and 'clean-voided urine from men was distributed into two distinct groups one group with 0—10^4 colonies/ml urine, and another group with more than 10^5 colonies/ml urine

The latter group was made up of patients with urinary tract infection of unspecified location Only five per cent of the patients with known urinary tract infection had bacteriuria of less than 10^5 On the basis of these findings Kass sets 10^5 colonies/ml urine as the limit between significant bacteriuria and contamination This limit has since been confirmed and used by many other authors (9)

Comparable results were obtained by MacDonald et al (14) in their investigations on an autopsy series, entailing comparison of the pathological findings and the results of quantitative urine culture from a suprapubic bladder puncture performed at an unfixed interval post mortem

Effersoe and Jensen (4) carried out a similar investigation comparing the histopathological lesions in an autopsy series with the bacterial count of urine collected immediately post mortem by catheterization None of these patients had received chemotherapeutic treatment or had been catheterized before Their studies imply a lowering of the limit to 10^4

With the 95 per cent limit for evaluating the figure for the incidence of bacteriuria in the present normal material it is found that the boundary between significant bacteriuria and contamina

tion is in the region of 10^5 colonies/ml urine, as found by the other investigators mentioned

It appears from fig 3 that the bacterial count in women is shifted towards higher values than found in men It might be considered justified to lower the limit for significant bacteriuria in men to 10^4 or below, but the present material is not sufficiently large to allow such a decision

Summary

On a material of 175 patients with healthy kidneys, taken from a casualty room the author's method, as described in detail, indicates that the limit between significant bacteriuria and contamination lies near 10^5 colonies/ml urine It is observed that the bacterial count in women is often higher than in men

Acknowledgement

This study was supported by grants from Svenska Diabetesförbundets forskningsfond and NOVO fonden

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From the III Medical Department (Head F Raaschou M D) Kommunehospitalet
Copenhagen Denmark

Studies on Urinary Infection in Diabetics

I Bacteriuria in Patients with Diabetes Mellitus and in Control Subjects

By

RENE VEJLSGAARD

The incidence of urinary tract infections in diabetics was formerly considered to be 3—4 times higher than in non diabetics but investigations in recent years by means of quantitative urine culture have cast doubt on this claim The aim of the present study was to examine the classic assertion that diabetic patients contract urinary infections more frequently than non diabetics, by investigating a large series of out patients with diabetes and a comparable group of controls with a method for urine collection involving little accidental contamination and with quantitative urine culture

Previous investigations have been concerned with autopsy studies (1 2 3 8 12 17, 30, 32, 37) and biopsy findings (5 34) and with uncontrolled clinical studies (10 14, 20 24 33) these are mentioned elsewhere (36) Only clinical studies will be presented here

Table I shows the incidence of urinary tract infections evaluated by various criteria, in patients with diabetes mellitus and in control groups

These studies show considerable variation some being frankly contradictory even when the investigators have used the same criteria for the presence of urinary tract infection Reasons for this variation include poor or selective choice of control group, and differences in sex and age between groups, the picture thus being distorted Other exclude patients with urological disease from the control group A few omit to state the composition of the material as to basic disease age etc, thus making the evaluation difficult Most authors fail to provide information of the criteria for exclusion of diabetics from the control material

An important reason for the discrepancy between the investigations is the varying condition of the patients at the time of investigation whether they were out patients or hospitalized or how long after admission the examination took place

It does not appear reasonable to compare a group of out patient diabetics

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tion is in the region of 10^5 colonies/ml urine, as found by the other investigators mentioned

It appears from fig 3 that the bacterial count in women is shifted towards higher values than found in men It might be considered justified to lower the limit for significant bacteriuria in men to 10^4 or below, but the present material is not sufficiently large to allow such a decision

Summary

On a material of 175 patients with healthy kidneys, taken from a casualty room, the author s method, as described in detail, indicates that the limit between significant bacteriuria and contamina tion lies near 10^5 colonies/ml urine It is observed that the bacterial count in women is often higher than in men

Acknowledgement

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31 Wilson P W & Kulemann E O J Bact *22* 71 1931

tients R chosen randomly S selected C catheter specimen V urine voided under sterile

Non-diabetics

Category	Sex	Average age	Number of pat	Incidence of bact uria (%)	Criteria of infection	Urine sample
1 obese	♀		24	75	Pos cultiv	Bladder
	♂ ♀	~58	50	8	Mic of sed	C-♀ V ♂
S	♂	56	150	59	Pos cultiv	C ♀
	♀	60	130	43		MS ♂
I R.	♀		20	10	Pos cultiv	C
O R	♂		102	4	$>10^5$ col	
	♀		337	6	/ml urine	C
I R.	♂		9	22	$<5\times10^4$ col	MS
	♀		41	26	/ml urine	
I S	♀	38	141	52	Pos cult	C
I R	♂	55	300	8	Mic of sed	
	♀	51		12	bact pyuria	
I S	♂		84	~5	$>10^4$ col	MS ♂
	♀		116		/ml urine	C-♀
O R	♂ ♀		150	12	$>10^5$ col	MS ♂
					/ml urine	C-♀
O	♂ ♀		99	7	>10 col /ml urine	MS

Material

During the period of investigation from 1 February 1962 to 31 August 1962 all patients examined consecutively in Hvidore Hospital for Diabetics and found to have verified diabetes mellitus were included in the material

A total of 269 patients were examined Fig 1 shows sex and age distribution The average for men was 44 4 years for women 31 1 years The ratio men to women was 1 1 1

The control material for this investigation is defined as a population in absolute con cordance with the chosen population of diabetics the sole criterion of exclusion being diabetes mellitus which could be diagnosed

Thus no patient in the control material had a post alimentary venous blood sugar level over 130 mg/100 ml or glycosuria or a family history of diabetes mellitus If only one of these criteria was satisfied, the individ ual was excluded from the material

The controls were selected from among the cases of minor accidents attending the casualty department of Kommunehospitalet during the period 1 May 1962 to 15 September 1962

A total of 260 patients satisfied the established criteria Their sex and age distribution is seen from fig 1 The mean age for the men was 38 6 years, for the women 38 0 years The ratio of men to women was 1 2 1

Methods

All the persons participating in the study were questioned and examined by the author Urine was collected by mid stream technique

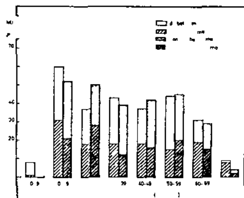


Fig 1 Sex and age distribution in diabetic and non diabetic series

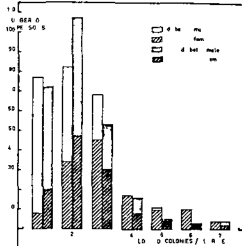


Fig 2 Incidence of bacteriuria in diabetic and non diabetic series

In the case of the women the same nurse assisted in its collections at the two places mentioned while in the case of the men assistance and instruction was provided by the author The technique has been described elsewhere (35) All specimens were obtained within four hours after last micturition

Quantitative urine culture was done with a modified flood plate method as previously described (35) The results are averages of counts on three plates All the bacteriological work was done by the author The urine samples were coded in such a way that the identity of the patients was unknown to the investigator during bacteriological testing

Blood sugar was determined according to Hagedorn Norman Jensen s method (13)

Qualitative urinalysis for glucose was done with Clinistix® and quantitative analysis was done by polarimetry

Results

The main results appear in fig 2, which shows the numerical distribution of bacteria/ml urine

Significant bacteriuria, i e more than 10^5 colonies/ml urine is recorded in table II in relation to sex Evidently there is no significant difference in the incidence of bacteriuria between diabetic and non diabetic males

In diabetic females, on the other hand the incidence of bacteriuria is higher than in non diabetic females With the X^2 test it is found that $0\,05 > p > 0\,01$

TABLE II Percentage of urinary infections in relation to sex in diabetic and non diabetic series

Sex	Diabetics Total no	$>10^5$ col /ml urine		Non diabetics Total no	$>10^5$ col /ml urine	
Males	141	1	0 7 %	146	3	2 1 %
Females	128	24	18 8 %	114	9	7 9 %
Total	269	25		260	12	

Plotting of the percentage distribution of bacteriuria against age, as in fig 3, reveals no significant difference in the incidence of bacteriuria between older and younger subjects, the boundary being drawn at 40 years such that the material falls into almost equal groups

It was of interest to correlate the incidence of bacteriuria in diabetic patients with the presence of other conditions or diseases which are known to involve a raised incidence of significant bacteriuria It was also sought to elucidate the incidence of asymptomatic bacteriuria in diabetics, and the possible influence of previous disease of the urinary tract

The results are given in table III

A record of dysuria was required if the case were to be classified as one with current urological complaints A history of past urological complaints was considered positive when the patients had been admitted to hospital for urinary tract complaints at some previous time,

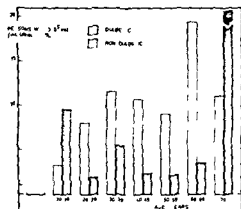


Fig 3 Urinary infection in relation to age in the diabetic and non-diabetic series

TABLE III Urinary infection in relation to present and previous urinary symptoms

	Diabetics			Non diabetics		
Urinary symptoms	Total no	>10⁵ col /ml urine		Total no	>10⁵ col /ml urine	
Present	8	3	37 5 %	3	0	0 %
None present	261	22	8 4 %	257	12	4 7 %
Previous	69	11	15 9 %	42	5	11 9 %
Non previously present	199	14	7 0 %	218	7	3 2 %

TABLE IV Urinary infection in relation to previous instrumental manipulations of the urinary tract

	Diabetics			Non diabetics		
	Total no	>10⁵ col /ml urine		Total no	>10⁵ col /ml urine	
Performed	74	11	14 9 %	39	3	7 7 %
Not performed	194	14	7 2 %	221	9	4 1 %

TABLE V. Urinary infection in relation to anaemia

Hb concentration	Total no.	$>10^5$ col./ml urine	
≥ 126 g/l	245	20	8.2 %
≤ 125 g/l	19	5	26.3 %

TABLE VI. Urinary infection in relation to ESR

ESR (mm/h)	Total no.	$>10^5$ col./ml urine	
< 9	146	6	4.1 %
10–19	68	8	11.7 %
20–39	24	4	16.7 %
>40	14	6	42.9 %

or had been treated for these by their own physician.

It is seen that 22 out of 25 diabetics with bacteriuria (88 %) had no symptoms.

In table IV correlating significant bacteriuria to previous instrumental manipulations of the urinary tract, i.e. catheterization or cystoscopy for diagnostic or therapeutic purposes, it is observed that not only do diabetics undergo manipulations more frequently than non-diabetics, but also that diabetics with a history of previous catheterization show a possibly significantly higher incidence of bacteriuria than noncatheterized diabetics ($0.1 > p > 0.05$).

That a definite correlation emerged between significant bacteriuria and anaemia, defined as a haemoglobin concentration less than 125 g/l (table V) and between bacteriuria and hypersedimentation (table VI), may perhaps be regarded as re-inforcing the choice of 10^5 colonies/ml urine as the most meaningful limit.

An attempt to correlate bacteriuria to pregnancy shows no significantly greater frequency of bacteriuria in diabetic women who have had children than in diabetic women who have not, 20.6 % against 16.7 % with more than 10^5 colonies/ml urine. The non-diabetic material shows corresponding figures (8.9 % against 6.9 %). Nor has parity any influence on the figures.

With the chosen upper limit of 160/110 for normotension in this out-patient material, no significantly higher incidence of bacteriuria was found among patients with hypertension than among those with normal blood pressure, either in the diabetic or in the non-diabetic series.

In neither series was there significantly greater incidence of bacteriuria among obese patients. The fact that only very few of the urine samples from obese women gave growths reflects the reliability of the method of collecting urine.

The patients with significant bacteriuria are uniformly distributed over the groups covering the range of glucose excretion 0—60 g glucose/24 hrs. No patient with a urinary glucose secretion exceeding 60 g glucose/24 hrs., and no patient with ketonuria, had bacteriuria, probably because hyperosmolar and acid urine respectively are inimical to bacterial growth.

Discussion

For the present study it was felt essential that the material should reach the size

achieved, that the groups be unselected and perfectly comparable in regard to sex and age, that neither group be hospitalized, and finally that all clinical and bacteriological examinations be done by the same person

The diabetic material is hardly a representative sample of the general diabetic population in contrast to the material of O Sullivan et al (27), but it was considered important that the diabetic series should consist primarily of young diabetics, thus decreasing sources of error such as unreliable history, etc

The control group is regarded as representative of the general population, but it should be pointed out that even with the criteria employed, exclusion of possible patients in the pre diabetic stage would not be possible

In the present controlled study no correlation was sought between bacteriuria and season, occupation, or geographical location, as the literature shows with certainty and unanimity that bacteriuria is unrelated to these factors (14 23 26)

Pregnant women and patients who had received chemotherapy within the last week prior to examination were not excluded from the study This could hardly confuse the results, as these persons were three pregnant diabetic women and two pregnant non diabetic women small and almost equal numbers The same argument applies to patients under chemotherapy or in whom chemotherapy had just been withdrawn (7 diabetic patients against 5 non-diabetic patients)

With the criterion of 10^5colonies/ml urine for significant bacteriuria, and with no distinction between sexes the incidence of significant bacteriuria in diabetic out patients was found to be 9 3 % against 4 6 % in non diabetic out patients If these findings are compared with the results of other investigators also working with groups of out-patients and making no differentiation between the sexes, and furthermore employing the same limit for contamination (9,27), the values obtained in the present study are found to be lower in both series

It is reasonable to assume that the lower mean age in the present material plays a role in the difference found

Szucs et al (33) give lower percentage values than the present author which is surprising since they employ a lower value for the limit of contamination

The incidence found in ambulatory diabetic women, in comparison with non diabetic women, corresponds approximately to the results obtained by others (23)

The results are not comparable with those obtained by Huvos and Rocha (18) and Rengarts (29) as these series are composed exclusively of hospitalized patients

In the present study unlike other studies, it has not been possible to establish a correlation between significant bacteriuria and increasing age The reason for this discrepancy may be sought in the far greater number of aged patients examined by other authors Thus in Huvos and Rocha s series 76 % of the patients were over the age of 50 years whereas the corresponding percentage in the present study was 31 % O Sullivan et al had

more than 50 % of their patients over the age of 60 years, against 15 % in the present study

Of the diabetic patients with significant bacteriuria, 88 % were asymptomatic This value is somewhat higher than that found by Huvos and Rocha

The general conviction that diabetic patients undergo catheterization more frequently than non diabetic patients was confirmed, but it was not possible to demonstrate that catherenization had been a contributory factor in the significant bacteriuria As it was not possible to demonstrate such a relationship in the control material either, catheterization must play a minor role as a causal factor

The investigation of infections of the urinary tract in relation to pregnancies, hypertension and glycosuria has not provided any indication that these factors predispose to significant bacteriuria

The investigation has strengthened the assumption that diabetic patients have a greater incidence of significant bacteriuria than non-diabetic subjects In the case of women, the figures are shown to be *statistically significant* This is in agreement with general clinical experience that diabetic patients have a higher incidence of infections than non diabetics and, in the case of diabetic women, a higher incidence of infections of the urinary tract It should be stressed, however, as has been done elsewhere (7), that when diabetes mellitus is provoked by alloxan treatment or pancreatectomy, no change occurs in infection resistance (28, 31) Hamsters with spontaneous diabetes mellitus show only few morphological signs of infection

Reduced resistance to infection could be demonstrated only throughout the acute toxic stage occurring shortly after the ingestion of alloxan in rabbits It appears to be the toxic rather than the diabetogenic action of alloxan which is responsible for this (31)

A set of autopsy statistics (30) shows that the incidence of all infections is the same in diabetic patients and non diabetics, except that pyelonephritis occurs with a significantly higher incidence in diabetic patients

There is therefore some support for the classic claim of an increased incidence of significant bacteriuria and for those factors generally considered as being responsible for the increased incidence

Summary

From autopsy studies, it has been shown that infections of the urinary tract are 3—4 times more common in diabetic patients than in non diabetic patients This claim has been questioned in the controlled clinical studies of recent years, in which quantitative bacterial culture from urine has been used in evaluating the results Usually, these studies have not disclosed significant differences, but they have shown much variation in material, methods and results

With an unselected out patient material of 269 diabetic patients and a strictly comparable group of 260 non diabetic, the following results were found

1 9 3 % of the diabetic patients had a bacteriuria with more than 10^5 colonies/ml urine, as compared with 4 5 % of non-diabetics The difference was not significant, but close to the 5 % level

2 When the sexes were considered separately, 0 7 % of the men had more than 10^5colonies/ml urine In the non-diabetic group 2 1 % had a bacteriuria with more than 10^5colonies/ml urine

3 In the case of the women, 18 8 % had more than 10^5colonies/ml urine In contrast, 7 9 % of the non diabetics had more than 10^5colonies/ml urine This difference is significant

4 Bacteriuria appears to have no relation to increasing age

5 Of the patients with bacteriuria, 88 % were asymptomatic

6 It is confirmed that catheterization is performed more frequently in diabetic patients than in non diabetic patients, but there does not appear to be any relationship between previous instrumental manipulations of the urinary tract and significant bacteriuria

7 A statistical relationship has been demonstrated between significant bacteriuria, anaemia and hypersedimentation

8 No correlation is found between significant bacteriuria and parity, arterial hypertension or the degree of glycosuria

The conclusion is that the classic claim of an increased incidence of urinary infection in diabetes, as judged by significant bacteriuria holds for women suffering from diabetes but the pathogenesis is obscure

Acknowledgement

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Addendum

After the conclusion of this manuscript a similar investigation has been published by R Osterby Hansen Acta med scand 176 721 1964

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Studies on Urinary Infection in Diabetics

II Significant Bacteriuria in Relation to Long term Diabetic Manifestations

By

René Vejlsgaard

The aim of the study has been to examine whether significant bacteriuria defined as a bacterial count greater than 10^5 colonies/ml urine can be correlated with vascular disease associated with diabetes of long duration

Consideration is also given to the frequency of significant bacteriuria in relation to the duration of the diabetes, as well as to age at onset of the disease and its degree of severity

Material and methods

The material consists of an unselected non hospitalized group of patients with well diagnosed diabetes mellitus The composition of the material is discussed in detail elsewhere (8)

The distribution of the patients according to duration of the diabetes is shown in fig 1 The duration varies from 0 to 51 years with a mean duration of 11 6 years for men and 12 0 years for women 31 6 per cent had a duration of disease of more than 15 years

The anamnestic and clinical examination of all the patients as well as the bacteriological work was performed by the author The procedure for collecting the urine and the bacteriological methods used have already been discussed (9)

Ophthalmoscopy was carried out in mydriasis, with two examiners for each In cases showing pronounced lesions photography of the retina was performed

Proteinuria was determined by Heller s method

The neurological status was evaluated on the basis of an objective neurological examination and a determination of the vibratory sense measured by biothesiometry

The cardiac state was evaluated by both objective and subjective criteria (angina pectoris tendency to oedema functional dyspnoe and previous incidents of coronary occlusion)

The peripheral vascular status was evaluated on the basis of objective vascular examination and oscillometry

The χ^2 test was applied in the statistical analysis

Results

Duration of diabetes

Fig 2 shows the percentage distribution of diabetics with significant bacteriuria in relation to the duration of the diabetes

Submitted for publication June 8 1965

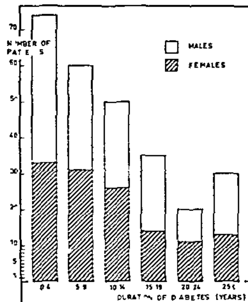


Fig 1 Distribution of diabetic series according to duration of diabetes

The figure shows that for the 5 year groups chosen, and with a duration of diabetes up to 19 years, no great difference is found in the incidence of bacteriuria

If the boundary between long and short duration of diabetes is placed at 10 years, as has been done by previous investigators (1, 2), and as would be natural in view of the mean value found in the present study, no significant difference is found between the groups having durations less than and more than 10 years ($p > 0.1$)

Neither is any significant difference found if the boundary is placed at 15 years ($p > 0.1$)

If the boundary is placed at 20 years, it is found that the difference in the incidence of bacteriuria is probably significantly greater in the group with the longer duration of diabetes, ($0.1 > p > 0.05$)

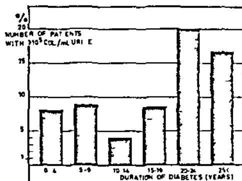


Fig 2 Incidence of urinary infection in relation to duration of diabetes

Onset of diabetes

If the distribution of patients with a bacterial count of more than 10^5 bacteria/ml urine is examined in relation to the age at onset of the disease, the percentage incidence of bacteriuria is seen to increase with increasing age Thus, the frequency is 7 % (8 out of 121 patients) with an age at onset between 0—19 years, 10 % (8 out of 80 patients) with an age at onset between 20—39 years, 12 % (7 out of 57 patients) with an age at onset between 40 and 59 years If the patients were over the age of 60 years at the onset of diabetes, the frequency of the bacteriuria increased to 22 % (2 out of 9 patients)

Diabetic retinopathy

The following criteria were chosen in evaluating this condition

Grade 0 represents normal ophthalmoscopy, grade I shows a few scattered microaneurisms grade II shows numerous microaneurisms (10) together with haemorrhages and exudates Grade III represents progressive proliferative retinopathy

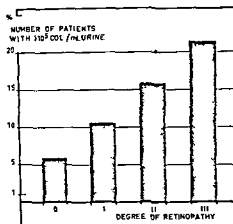


Fig 3 Urinary infection in relation to diabetic retinopathy

A total of 153 patients were found with grade 0 (58 5 % of the whole material), 38 patients with grade I (14 5 %), 57 patients with grade II (21 8 %) and 14 patients with grade III (5 3 %)

Fig 3 shows the percentage distribution of urinary infections in relation to the degree of the ophthalmoscopic findings As the degree of retinopathy increases it is accompanied by an increasing incidence of urinary infections Patients with retinopathy covered by grades I—III have a significantly greater incidence of bacteriuria than patients without retinopathy, ($0 05 > p > 0 01$)

If patients with retinopathy of grades II—III are compared as a group with patients with retinopathy of grades 0—I, a significantly higher incidence of bacteriuria is found ($0 05 > p > 0 01$)

No striking differences can be found on analyzing the age groups within the individual grades of retinopathy

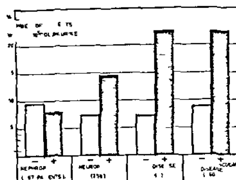


Fig 4 Urinary infection in relation to various disorders in diabetes mellitus

Diabetic nephropathy

Constant proteinuria was taken as the *criterion for the presence of nephropathy* Patients showing transitory proteinuria were classified as normal

Of the 267 patients examined 25 had proteinuria (9 4 %) two of them (8 %) with significant bacteriuria, while 23 of the remaining 242 patients without proteinuria had bacteriuria (9 5 %)

Diabetic neuropathy

The criterion for the presence of neuropathy was disappearance of or diminution in the vibratory sense in relation to the state regarded as normal for that age (3)

A total of 83 patients showed neuropathy *corresponding to 32 % of the total* number of patients examined Twelve of the patients found to have neuropathy (14 5 %) had bacteriuria with a bacterial count greater than 10^5 colonies/ml urine, while 13 (7 4 %) of the other 176 patients had bacteriuria

The incidence of infections of the urinary tract is however, not significantly higher in diabetic patients with neuropathy

TABLE I Urinary infection in relation to severity of diabetes

Insulin dose (I U /24 h)	Total no	$>10^5$ col /ml urine	
0	9	2	22 2%
<15	19	2	10 5%
16–30	57	5	8 8%
31–45	123	10	8 1%
46–59	44	3	6 8%
>60	25	3	12 0%

Heart disease

A patient satisfying merely one of the following points was classified as suffering from coronary sclerosis a) an abnormal ECG, b) typical angina pectoris, previously diagnosed cardiac infarction, c) objective signs of cardiac insufficiency

None of the patients in the material provided any anamnestic support for the presence of heart disease of other origin

Coronary sclerosis was found in 29 patients corresponding to 10 9 % of the total material Of these 8 (27 6 %) had urinary infection, while the corresponding percentage in diabetic subjects without heart disease was 7 2 % (17 out of 236 patients)

The figure for the incidence of bacteriuria among diabetics with coronary sclerosis is significantly high

It is worth mentioning that 6 out of 8 patients with heart disease and a bacterial count greater than 10^5 colonies/ml urine had retinopathy of grade III Only one of the 6 had proteinuria

Peripheral vascular disease

If an oscillometric deflection of less than 1 was recorded the patient was considered to be suffering from peripheral vascular disease

This group consisted of 11 patients 4 9 % of the total Three of these patients (27 %) had urinary infection

In the group showing a deflection more than one 9 % (22 out of 249) had significant bacteriuria greater than 10^5 colonies/ml urine

Degree of severity of diabetes

This is evaluated from the insulin dose on the day of examination Table I shows the classification as well as the results The degree of severity does not appear to have any influence of the incidence of significant bacteriuria

Discussion

In a preceding study (8) on the incidence of bacteriuria in a group of unselected young diabetic subjects, in relation to the incidence in a comparable group of the general population, but without diabetes, it was shown that diabetic subjects — with statistically significant values for women — have a higher incidence of significant bacteriuria A further evaluation of this material shows that there was no difference in the incidence of significant bacteriuria between groups of patients with a duration of diabetes less than 10 years and more than 10 years, respectively This finding is in line with the results of previous investigations (1 5)

Possibly, however, significant bacteriuria may occur more frequently in patients with a duration of diabetes longer than 20 years as the p value is close to the 5 % level

Rengarts (5) is the only investigator who has previously attempted to relate urinary infection to vascular disease in diabetes of long duration Among dia betic subjects with a bacterial count greater than 10 colonies/ml urine, he finds 26 % with diabetic retinopathy but no further details are given

In the present study it was possible to show a significant increase in incidence of bacteriuria with increasing grade of retinopathy

A corresponding correlation between diabetic neuropathy and bacteriuria, and between diabetic nephropathy and bacteriuria, could not be demonstrated The vague criteria are presumably responsible for the inability to establish such a relationship Reduced vibratory sense and proteinuria respectively, have been used as measures of the presence of disease these being symptoms, which are known to have their onset subsequent to the real onset of the disease Thomsen (7) for example, has shown that proteinuria is not observed until a considerable period after specific diabetic lesions can be demonstrated in the glomeruli This problem might be solved through com parison of biopsy findings with culture results Steiness (6) has shown corre spondingly, that reduced vibratory sense is not an initial *symptom in diabetic* neuropathy

If significant bacteriuria were asso ciated with the syndrome of diabetic angiopathy (4) it should likewise show a correlation with duration of diabetes, but this has not been shown with certainty in the present study

In the present study urinary infec tions (i e significant bacteriuria) were definitely linked with the most specific diabetic vascular disease, retinopathy, although, it does not follow that there is an association with diabetes mellitus per se

The present study considered in conjunction with previously published investigations thus strengthens the prob- ability that diabetic vascular disease is a contributory factor in the development of urinary infections in diabetic patients

This relationship, together with the conception of significant bacteriuria being dependent on vascular insufficiency and on neurological disorders in bladder-function suggests the desirability of further pathological studies on the question

Summary

Quantitative bacterial culture from mid stream urine was carried out on an unselected, non hospitalized material of 269 diabetic patients with 10 colonies/ml urine as a criterion for the presence of urinary infections The findings were as follows

1 Urinary infections cannot with certainty be correlated with increasing duration of diabetes

2 The incidence of urinary infections *increases significantly as retinopathy* becomes severer

3 Urinary infections cannot be corre lated with diabetic nephropathy or with diabetic neuropathy This is explained by the inadequate criteria employed for these diseases in the present investiga tion

4 The incidence of urinary infections increases with increasing heart disease

coronary sclerosis and peripheral vascular disease

5 The severity of diabetes as evaluated by insulin requirements, appears to have no bearing on the incidence of urinary infections

6 It appears that urinary infections cannot be correlated with diabetes mellitus per se, but the present study suggests that diabetic vascular disease is a contributory factor in the development of urinary infections in diabetic patients

Acknowledgement

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Scleroma in Sweden

By

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Scleroma previously called rhinoscleroma, is a disease which is rarely seen in Scandinavia in spite of the fact that endemic areas are situated not far from our boundaries As it seems likely that it will be encountered more frequently in the future, a short description of the illness and a report of two cases were felt warranted

The clinical picture of scleroma was first described in 1870 by the dermatologists von Hebra and Kaposi Twelve years later von Frisch demonstrated a gram negative rod, Klebsiella rhinoscleromatis, in the lesions Though this bacillus can invariably be cultured from untreated cases of scleroma opinions still differ as to whether it is the cause of the disease (19) In 1909 Goldzieher and Neuber (4) described a complement fixation test using as antigen an extract of Klebsiella rhinoscleromatis The test proved positive in 80—90 % of patients with scleroma The disease is not common anywhere (about 4 000 cases have been reported in the literature) but it occurs mainly in eastern and central Europe (20) and in South and Central America (15) It spread during and after the world wars I and II Minor groups of cases have occurred in various parts of the world and a few cases have been seen in Sweden (2, 6 18) In endemic areas it is above all the lower social classes that are affected particularly between the ages of 15 and 35 years The disease is believed to be spread by aerial or contact infection, but its contagiosity is only weak

Scleroma usually appears initially as rhinitis, first catarrhal later atrophic, followed by the formation of granulomas in the nasal mucosa where inflammatory cells can be demonstrated, especially plasma cells After some time these granulomas assume a cartilaginous consistency and the microscopic picture shows the characteristic Mikulicz-cells and Russel s bodies In the later stages of the disease the picture is dominated by formation of scar tissue The changes may spread within the airways the nasal sinuses the gums the pharynx the tongue and the larynx may also be involved and the disease may also, though less often start in one of these

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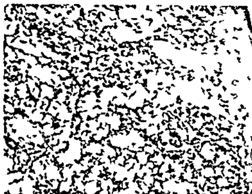


Fig 1 Case 1 Biopsy from the nasal mucosa The surface epithelium to the right shows squamous metaplasia The underlying tissue contains numerous cells of Mikulicz

regions Occasionally the disease spreads to the upper lip, or to the skin over the nose and gives the patient a characteristic appearance ('Hebra nose') The lesions sometimes assume monstrous proportions The nasal cavities are gradually obstructed, the sense of smell is lost and laryngeal changes fairly often make tracheotomy necessary The cervical and paratracheal lymph nodes may also be involved Spread to the cranial cavity (fossa anterior) has been reported, though rarely (7) The disease runs a protracted course and the prognosis *quo ad vitam* is good sudden death may, however occur because of obstruction of the respiratory tract (8, 16) Supervening infection with syphilis or tuberculosis has been described as has suspected malignant transformation Secondary bacterial infection of the lesions is common

Treatment has varied roentgen radiation, antimony preparations and treatment with a vaccine made from the patient's own Klebsiella strain have been used with some success but since 1946 when streptomycin was first used in the treatment of the condition (14), this antibiotic has become the drug of choice Other antibiotics, such as chlortetracycline, tetracycline and chloramphenicol have also proved useful In some quarters steroids are given initially together with streptomycin Surgery is indicated in cases with obstruction of the airways and plastic surgery may be indicated after the disease has been cured, but during the active phase of the disease surgical intervention involves the risk of spread of the lesions and deforming scars This also applies to roentgen treatment of scleroma

We have had the opportunity of seeing two patients at Lund hospital and Orup's Sanatorium Those two cases are reported below

Case reports

Case 1 A woman, aged 40 from Croatia where scleroma is endemic She came to Sweden as a refugee in 1963 As far as she knew her parents and siblings were healthy She had been admitted to hospital on one or two occasions in her home country because of mental depression and some time after her arrival in Sweden she was admitted to hospital because of a temporary confusion presumably precipitated by stressing environmental conditions

She had been operated upon in Zagreb about 1956 because of chronic obstruction of the nose Nothing is known about any other symptoms she might have had She had probably not been treated with any drugs She did not return to the hospital for follow up and a few years later the same type of symptoms recurred In the summer of 1963 she was repeatedly examined at the ear nose throat department Lund hospital (Ingelstedt Hoorn A Lundgren) The patient's nose was moderately deformed

being widened and somewhat saddled Both nasal cavities were narrowed by scars and especially the right cavity was obstructed by firm tumour masses Similar changes were seen in the epipharynx with cicatricial contraction and granular formations in the roof The rest of the pharynx the larynx the oral cavity and the lips showed nothing remarkable Skull X ray revealed no abnormalities apart from a tumour like mass in the nasal cavity and narrowing of the choanae — Scleroma was suspected and a biopsy specimen was removed Microscopic examination showed a typical picture of scleroma as well as granulomatous fibrous changes (fig 1) Culture of nasal secretion and of biopsy material gave growth of Klebsiella bacteriae In addition the complement fixation test for Klebsiella rhinoscleromatis was positive

In July 1963 the patient was referred to Orup s sanatorium for long term antibiotic treatment On admission she was tired and appeared somewhat thin No palpable lymph nodes Chest X ray normal Afebrile E S R 20 mm/l hour Slight anaemia (Hb 11 g/100 ml) and polynuclear leucocytosis (9 700/ mm³) Mantoux positive Renal function normal Wassermann s reaction was negative

The patient s strain of Klebsiella proved to be sensitive to tetracycline and streptomycin The patient was first treated with parenteral rolitetracycline (Syntodecin®) 0 35 g daily for 3 weeks and then with oxitetracycline 1 g daily by mouth for 1 month She was then given streptomycin in a dose of 1 g daily the total dose being 40 g Treatment was then stopped for psychological reasons No complications of antibiotic treatment were observed

Already during the first month of treatment the patient improved and the local symtoms regressed her general condition improved and the laboratory values became normal On rhinologic re examination 4 weeks later the granulations in the nose and epipharynx were found to have diminished and culture of the nasal secretion no longer gave growth of Klebsiella The improvement progressed for a further 2 months after which her condition became stationary At re-examination at the E N T department a few cicatricial patches that had not responded to antibiotic therapy were removed After about 5 months treatment the patient was discharged symptom free with satisfactory patency of the left nasal cavity She was then in good general condition She had put on 10 kg E S R normal Blood picture normal Histopathological examination now showed only non specific chronic inflammation with fibrosis Serological re examintion was made in March 1965 the patient then had a considerable lower titre in the complement fixation test

Case 2 A hitherto healthy farmer from the district of Linkoping, born 1928 He was married and had two healthy children No contact with persons with scleroma was known and he had not been abroad The condition appeared in 1955 with nasal obstruction and watering of the eyes Penicillin and symptomatics had no effect Some months later pea sized nodules appeared at the root of the nose In 1956 the nasal cavity was found to be filled by granulomatous tissue as were two maxillary sinuses and the right sphenoidal sinus Septal resection and operation ad modum Caldwell Luc was done Microscopic examination showed chronic inflammation without any specific signs and without evidence of tumour In December 1957 he complained of respiratory symptoms and at examination at the E N T -department Linkoping General Hospital 3 large tumours were found below the vocal cords and enlarged glands in the neck In April 1958 tracheotomy was done and since then the patient has a tracheostoma Histological examination (K Lindberg) suggested rhinoscleroma but the picture was not quite convincing — In May—September 1958 steroid treatment was given In 1959 further progression was noted and on histopathological examination Hodgkin s disease was suspected Roentgen treatment was given without any demonstrable effect In 1961 a biopsy specimen was removed P A D collagenosis? Repeated roentgen treatment was given with some effect after which the



Fig 2 Case 2 Tumour like masses in the root of the nose and the forehead

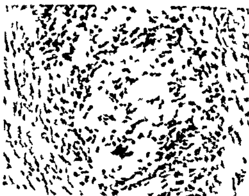


Fig 3 Case 2 Biopsy from the lesion on the forehead showing a granuloma centrally composed of large foamy histiocytes with a pale cytoplasm and an eccentric nucleus — probably Mikulicz cells Similar pictures were seen in biopsies from the nasal tracheal and bronchial mucosa cervical lymphnodes and from an infiltrate in the skin of the right arm

patient was given steroid treatment periodically In the spring of 1963 nodules appeared in both groins and the E S R (which had formerly been normal) was now 58 mm/1 hour Sternal puncture in November that years showed an increased number of plasma cells and lymphocytes

In January 1964 the patient was admitted to the Medical Department Lund University Hospital His general condition was good The most striking abnormality were large infiltrates in the root of the nose and in the forehead (fig 2) On the right lower arm the left thigh the left temporal region in both groins and on both sides of the neck similar firm glistring well defined subcutaneous growths were found They were adhering to the underlying surface not tender to palpation and their sizes varied from that of a pea to that of a plum On examination at the E N T -department (Ingelstedt Örtegren Flisberg) the nasal cavities were found to be obliterated by tumour masses which also obstructed the subglottis Bronchoscopy revealed pedunculated readily bleeding granulations at the origin of the left main bronchus Roentgen examination (tomography) of the facial skeleton showed alternating areas of destruction and sclerosis E S R 85 mm/1 hour Hb 11 2 g/100 ml leucocyte count normal with strong preponderance of neutrophil cells Sternal puncture shift to the left of myelopoietic series slight eosinophilia and possibly an increased number of plasma cells Renal and hepatic function normal Serum electrophoresis alb 3 87 α_1 0 35 α_2 0 67 β 0 99, γ 2 52, total protein content 8 4, thus decreased albumin and increased globulin content particularly increased γ globulin and to a lesser degree α_2 and β globulin fraction — WR Meinicke and VDRL were negative as well as the complement fixation tests for blastomycosis and histoplasmosis No growth of fungi was obtained in culture from the nasal mucosa Cerebrospinal fluid cells, sugar and protein normal microscopic examination, culture and animal experiments for cryptococcosis proved negative Mantoux positive to 1 mg Culture of a biopsy of a tumour for tubercle bacilli gave no growth Bacterial culture of material from the nose and from the tumour proved negative for Klebsiella but the complement fixation test for rhinoscleroma antigen was positive — The micro-

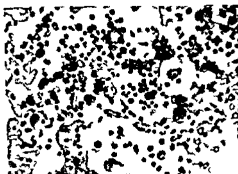


Fig 4 Case 2 Fine needle aspiration biopsy

scopic picture could fit with the diagnosis of scleroma (*fig 3*)

Fine needle aspiration biopsy (N Soderström) yielded cell rich smears with a rather peculiar type of granuloma cytology which was similar in specimens from different lesions of the process (frontal and nasal tumours para auricular lymph node infiltrate on right fore arm) The cell picture was very polymorphous with a mixture of tissue bound phagocytes plasma cells scattered epitheloid cells eosinophilic leucocytes and tissue mast cells Polynuclear giant cells were numerous at a first glance similar to Reed Sternberg cells but with small and distinct nucleoli and usually containing phagocytized material A unique feature was the abundant occurrence of compressed spherical lumps of seemingly viable lymphocytes in some cases obviously enclosed within large cells of reticulo-endothelial type (lymphocyte emperipolesis — fig 4) The *smear* cytology of scleroma has not been described hitherto but the fine photomicrographs published by Lennerth (10) show a picture very similar to that found in the present case

Treatment with streptomycin was started and continued until the patient had received altogether 60 g because of *inter alia* allergic reactions streptomycin was then withdrawn and an attempt was made to check the granuloma proliferation with cyclophosphamide (Sendoxan®) On readmission in January 1965 the appearance of the tumours was largely the same as previously The complement fixation test was still positive Streptomycin therapy was resumed now without side reactions A total dose of 40 g was given in this second cure The case is still under observation

Serology The complement fixation test was performed with a Klebsiella rhinoscleromatis strain obtained from The International Escherichia Centre in Copenhagen The antigen used was a heat killed 24-hour agar culture in physiological saline with a density of approximately 3 mill bact/ml A modification of the test described by Guerkesse and Aloukere (5) was first used 0 5 ml of the patient s serum diluted 1/5 was added to each of 5 Widal tubes to which were further added 0 5 ml complement and 0 5 ml neat antigen or antigen diluted 4/5 3/5 2/5 and 1/5 respectively — Later on the test was made in a manner similar to the routine Wassermann procedure the patient s serum was diluted 1/5—1/10—1/20—1/40 etc and the antigen was not diluted the proportions between antigen serum and complement were the same in both cases After 1 hour in the thermostate at 37 C the hemolytic system was added and after 30—45 minutes at 37 C the test was read

Sera from *case 1* during her stay at the hospital inhibited hemolysis in 3 4 or 5 tubes when the first modification was used this corresponded to a titre of 1/80—1/160 when serum was diluted (2nd modification) On re examination one year later this titre had fallen to 1/10—1/20 — Serum from *case 2* inhibited hemolysis in 2 3 or 4 tubes corresponding to a serum dilution titre of 1/40 No titre change was encountered on re-examination in January 1965

For control purposes 134 normal sera were tried 131 gave negative reactions while 3 were positive one of them with a full titre The resulting figure 2 25 % false positive is in good agreement with values given in the literature (11)

Discussion

During the last decade Sweden has received a fair number of immigrants from countries known to have endemic

scleroma (Hungary, Jugoslavia) It is therefore not surprising to encounter the disease in such immigrants The first case is a typical example with characteristic symptoms, site and course and in which the diagnosis was confirmed histopathologically, serologically and bacteriologically The case illustrated that a relatively good effect of antibiotic therapy sometimes may be obtained even in advanced cases of the disease

In the second case we regard scleroma as the probable diagnosis in spite of several atypical features We base this opinion on the characteristic histologic picture and the positive complement fixation test No exposure was known, sporadic idiopathic cases in non endemic areas have, however, been described on several occasions (1, 3, 7, 17) The clinical and morphological picture in this case must have been blurred by roentgen treatment given already one year after the onset of the disease and by later steroid treatment It is true that scleroma had been assumed in 1958, but the microscopical picture on later occasions was equivocal, and in the next years malignancy or collagenosis were considered as possible alternatives The failure of our attempts to culture Klebsiella from the lesions may be explained by the fact that the patient was receiving antibiotic treatment at the time when most of the biopsy specimens were removed — More difficult to reconcile with the diagnosis of scleroma is the presence of multiple lesions outside the respiratory tract in this case, such spread has, as far as we know, not been described before in the literature, apart from a single case with Mikulicz cells in submucous foci in the intestinal mucosa in a patient with scleroma (16) The multiple foci in this case seem to speak against the diagnosis scleroma, in the light of our knowledge of this disease, but is on the other hand very interesting

The advanced scleroma may simulate neoplasm (cancer, sarcoma, Hodgkin's disease), or specific infections (tuberculosis, syphilis, leprosy, rhinosporidiosis) In the early, atrophic stage the condition may be confused with ozena or, according to Quevedo, chronic diphtheria (15)

Summary

Scleroma (rhinoscleroma) is rare in Scandinavia which is, however, not so very far from endemic regions in eastern Europe A short description is given of the clinical picture and the treatment of the disease Two cases are reported A woman from Jugoslavia had a typical clinical picture, the diagnosis could be verified by finding of Klebsiella bacteriae, and treatment with tetracyclines and streptomycin produced improvement The second case was seen in a native Swede who had not been abroad, since 1955 he had increasing tumour masses in the nasal cavities and trachea, over the bridge of the nose and, in addition, a few subcutaneous nodules in the groins and on the limbs Histological examination in both cases revealed findings compatible with a diagnosis of scleroma, and the complement fixation test was positive

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Primary Pulmonary Hypertension with Emphasis on its Etiology and Treatment

By

O Storstein, L Efskind, C Muller, R Rokseth and S Sander

Since the introduction of the technique of right heart catheterization the entity of primary pulmonary hypertension has been well defined from both clinical and hemodynamic viewpoints (6 7, 9 11, 15, 16, 25, 30) Much, however, remains to be learned about this disease, especially as to its etiology and treatment The prognosis in this condition is poor, the disease unrelentingly leading to death in the course of 1—10 years

Material and methods

During the last 6 years we have collected 17 cases of primary pulmonary hypertension from 1 350 right heart catheterizations carried out in the Cardiological Laboratory during the same period The patients have been subjected to right heart catheterization with determination of intracardiac pressures and of cardiac output using the Fick principle Respiratory studies have been carried out in most of the patients Some of the patients have been subjected to physiological studies to test the reactivity of the pulmonary vasculature In some patients direct determination of arterial PO_2 has been carried out before and after the operative procedure to be reported on later

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Results

Table I shows the sex and age distribution of the material There are 7 men and 10 women The age of the patients varies from 7 to 70 years There is an accumulation of women of the age group of 31—40 years Apart from this accumulation there is a fairly even distribution of age The duration of the illness varied from 1 to 22 years

The most common symptom was dyspnea on exertion found in all patients The dyspnea varied from slight dyspnea on effort to dyspnea even at rest There was, however no instance of nocturnal dyspnea Cyanosis was the next common symptom It was found in 13 patients The cyanosis usually was noted on exertion Only a few patients were cyanotic at rest Syncope on exercise is a common symptom in this disease It was found in 8 patients The mechanism of the syncope is supposed to be the same as that in aortic stenosis and pulmonic stenosis a critical reduction of the cardiac output with a reduced oxygen transport to the

TABLE I History

Name	Sex	Age	Duration of illness (yrs)	Dyspnea	Chestpain	Syncope	Cyanosis	Edema	Hemoptysis	Pneumonia	Thrombosis	Childbirths	Anticoagulants	Died
E K	♀	7	5	+	−	+	+	−	−	−	−	−	−	+
H.A	♀	45	20	+	−	−	−	+	−	−	−	7	−	+
B E	♀	39	5	+	+	+	+	+	−	−	−	2	+	
R F	♂	11	6	+	−	+	−	−	−	+	−	−	−	+
K.H	♀	36	20	+	−	+	+	+	+	−	−	−	+	
K T	♀	64	13	+	−	−	+	+	−	−	−	1	+	
M N	♀	31	1	+	−	−	−	−	−	−	−	−	+	+
A.P	♀	32	20	+	−	+	+	+	+	−	+?	1	+	+
E S	♂	43	13	+	+	−	−	−	−	−	−	−	+	+
S.Å	♀	55	5	+	+	−	+	−	−	−	+ cerebral	−	+	+
A B	♂	70	5	+	−	−	+	−	−	+	−	−	+	+
H J	♀	36	3	+	−	−	+	−	−	−	−	3	+	
E S	♂	52	1	+	−	+	+	+	−	−	−	−	−	+
R N	♂	62	2	+	−	+	+	−	−	−	+	−	+	+
B P	o	24	2	+	+	+	+	−	−	−	−	−	−	+
J F	♀	23	22	+	−	−	+	−	−	−	−	−	−	
P E	♂	58	2	+	−	−	+	+	−	−	+	−	−	+

brain Chest pain has been noted in 4 of our patients This kind of chest pain has been denoted hypercyanotic angina pectoris The pain is characterized by its relation to exercise and in this way it is similar to angina pectoris But the pain usually lasts longer, 15—30 minutes and there is only a slight response to nitroglycerol Edema was noted in 7 of the patients and hemoptysis in 2 pa tients

A history of previous thrombosis or embolism was noted in 4 patients Two patients had previously suffered from pneumonia

On physical examination (table II) cyanosis was the most common finding being observed in 13 patients No patient had clubbing of the fingers Most of the patients presented a systolic murmur usually of grade 2—3/6 at the upper left sternal border A diastolic murmur of the Graham Steell variety in the second or third left interspace was found in 6 of the patients The most pronounced sign on auscultation was an accentuated second pulmonic sound Usually it was heavily accentuated

Signs of right ventricular failure were found in 10 patients who had en

TABLE II Clinical findings

											Electrocardiogram	
Name	Sex	Age	Cyanosis	Clubbing	Systolic murmur	Diastolic murmur	Accent P_2	Liver	Edema	Sinus rhythm	Systolic overload right ventricle
E.K	♀	7	–	–	Grade 2–3	–	++	–	–	+	+
H A	♀	45	+	–	Grade 2–3	2 left interspace	++	+	+	+	+
B E	♀	39	+	–	Grade 3	2 left interspace	++	+	–	+	+
R.F	♂	11	–	–	Grade 2	2 left interspace	++	–	–	+	+
K H	♀	36	–	–	Grade 1	–	+	+	–	+	+
K.T	♀	64	+	–	Grade 2	–	+	–	–	+	+
M N	♀	31	+	–	Grade 2–3	3 sound	+	+	+	+	+
A.P	♀	32	+	–	–	–	++	+	–	+	+
E.S	♂	43	+	–	Grade 3	2 left interspace	+	+	–	+	+
S Å	♀	55	+	–	Grade 2	–	+	–	–	A V block	–
A.B	♂	70	+	–	Grade 2	–	+	–	–	+	+
H J	♀	36	+	–	Grade 2–3	2 left interspace	++	–	–	+	+
E S	♂	52	+	–	Grade 2–3	–	++	+	+	+	+
R N	♂	62	+	–	–	–	++	+	–	+	+
B P	♂	24	+	–	–	–	++	+	–	+	+
I F	♀	23	–	–	–	2 left interspace	++	–	–	+	+
P E	♂	58	+	–	–	–	+	+	+	+	+

largement of the liver, usually of slight degree Four of the patients also had edema

The electrocardiogram is characteristic in this condition, presenting a picture of systolic overload of the right ventricle (fig 1) Only 1 of the patients showed a different electrocardiogram He had a complete atrio ventricular block All the other patients were in sinus rhythm

Hemodynamic studies

The findings on cardiac catheterization are presented in table III We see that the picture is uniform The systemic blood pressure is normal or low The pulmonary artery pressure is greatly elevated, in some patients as high as or even higher than the systemic blood pressure It is well known that it is difficult to record the pulmonary arterial wedge pressure in these patients We succeeded in doing this in only 7 patients and in all of them the wedge pressure was normal The resting cardiac output was reduced in 12 of the patients while it was in the low normal range in the remaining 5 patients The pulmonary arteriolar resistance was invariably greatly increased in those patients where it

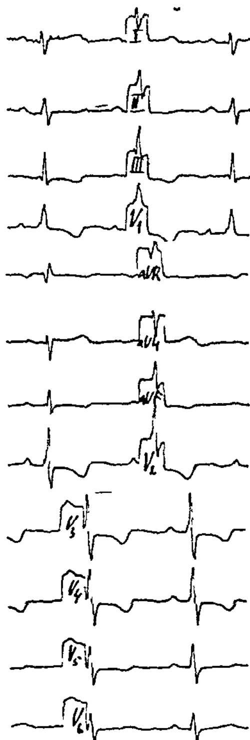


Fig 1 ECG showing systolic overload of the right ventricle in a case of primary pulmonary hypertension

was possible to calculate it The arterial oxygen saturation was usually found to be in the low normal range In some patients it was reduced, the lowest value being 79 per cent The arterial carbon dioxide tension was reduced in all patients due to the accompanying hyperventilation The oxygen capacity of these patients expressed as hemoglobin concentration was either normal or slightly increased

The ventilatory studies carried out in 10 of the patients invariably showed normal timed vital capacity ($FEV_{1\,sec}$) Maximal voluntary ventilation was normal in 8 patients, reduced in 2

In some patients hemodynamic studies were carried out to test the reactivity of the pulmonary circulation (table IV) In 2 patients an exercise test was carried out In one there was increase in pulmonary artery pressure during the exercise test The cardiac output was unchanged and there was a slight drop in arterial oxygen saturation In the other patient only the blood gases were studied during exercise and there was a distinct drop in arterial oxygen saturation

Four patients were tested with 100 % oxygen for 10 minutes The arterial oxygen saturation rose during this procedure, in one of the patients up to 100 per cent while the other 3 did not achieve full oxygen saturation of arterial blood

The effect of breathing 100 % oxygen, on pulmonary circulation was tested in 3 patients In 2 of the patients there was a slight drop in pulmonary artery pressure and a slight increase in cardiac output, indicating a slight reduction in

pulmonary arteriolar resistance In the third patient there was no change in pulmonary artery pressure and a fall in cardiac output with increase in pulmonary arteriolar resistance

One patient was tested with the adrenergic blocking agent Phentolamine Following 5 mg a drop in pulmonary artery pressure was observed after 4 minutes The same patient was later tested with the ganglion blocking agent Vegolysen Following 20 mg of this drug through the cardiac catheter there was a slight drop in pulmonary artery pressure The drop was less than after Phentolamine

Pathological studies

Pathological examinations have been carried out in 9 cases In 3 cases biopsy specimens obtained at thoracotomy have been examined Autopsy has been performed in 6 cases One of these patients A P has been reported on previously (22)

The characteristic pathological changes observed were pulmonary arteriosclerosis, medial hypertrophy of the muscular arteris and arterioles, a marked intimal proliferation with narrowing of the arterial lumen and occasional complete occlusion of the lumen by organized thrombi

The hearts examined were generally heavier than normal in our six autopsy cases the weight ranged between 425 and 610 g Hypertrophy of the right ventricle was invariably present the wall thickness varying from 3 to 12 mm

No associated congenital heart defects were found among our 6 autopsies

Atheromatosis was found localized to the great pulmonary arteries, a finding which is rare except in association with pulmonary hypertension The main pathological lesions were localized to the middle sized and small muscular arteries and to the arterioles The muscular arteries generally exhibited a prominent medial hypertrophy (fig 2) In small arteries, however, where the lumen was drastically reduced by intimal proliferation (fig 3) an atrophy of the media could occasionally be found

In four cases autopsy revealed recent pulmonary artery thrombosis superimposed on the lesions of pulmonary hypertension In one of these cases (E S) the thrombosis was associated with a large partly organized thrombus in the right atrium and in addition small thrombi were found localized to the right femoral vein Otherwise the origin of the thrombi remained obscure

No convincing signs of collagen disease or syphilis have been encountered in this study Our material do not give support to the view that angeitis localized to the pulmonary vessels are responsible for the lesion that cause raised pulmonary arterial pressure The concentric fibrous intimal thickening was found mainly in arteries where neither inflammatory nor necrotic lesions were present Only exceptionally did we observe restrictive changes and lymphocyte infiltration in the arterial wall with cellular connective tissue replacing elastic tissue and muscle fibers

The pulmonary venous system as well as the bronchial arteries was essentially normal

TABLE III Hemodynamic and respiratory studies

Name	Sex	Age	BP	PA	PAW	CI	PAR
E K.	♀	7	110/65	105/56	6	4 5	1,470
H.A	♀	45	135/85	50/31	—	1 9	—
B E	♀	39	130/80	95/50	—	1 9	—
R F	♂	11	110/80	61/44	3	2 6	1 252
K H	♀	36	120/80	100/47	2	2 3	1,364
K T	♀	64	155/85	83/39	—	1 7	—
M N	♀	31	100/70	75/38	3	1 9	1,100
A P	♀	32	110/85	125/55	—	3	—
E S	♂	43	170/90	110/5 (RV)	—	2 3	—
S.A.	♀	55	105/95	110/50	5	1 7	1 909
A B	♂	70	130/70	96/24	—	2 9	—
H J	♀	36	120/80	110/42	—	3 1	—
E S	♂	52	100/80	95/40	—	1 9	—
R N	♂	62	135/85	62/16	8	3 4	284
B P	♂	24	100/80	105/57	—	2	—
I F	♀	23	117/56	107/60	—	2 8	—
P E	♂	58	152/96	107/38	2	1 8	1,556

Abbreviations. BP blood pressure mm Hg PA pulmonary artery pressure mm Hg PAW pul resistance dynes/sec /cm⁻⁵ Sa_{O_2} arterial O_2 saturation per cent P_{CO_2} arterial CO_2 tension volume/1 sec MVV maximal voluntary ventilation lit/min RV right ventricular pressure mmHg

TABLE IV Studies of the reactivity of the pulmonary circulation

			BP		PA	
Name	Sex	Age	B	A	B	A
Exercise test						
A B	♂	70				
R.N	♂	62	—	—	62/16	100/40
100 % O_2 for 10 min						
A.P	♀	32	—	—	132/60	114/54
S.A	♀	55	—	—	110/45	110/2 (RV)
A B	♂	70				
H J	♀	36	—	—	103/43	91/38
Voluntary hyperventilation for 10 min						
E.S	♂	43	120/80	120/80	95/40	95/40
K H	♀	36	99/58	96/56	103/46	90/34
Phentolamine 5 mg—4 min						
B P	♂	24			105/57	83/53
Vegolysen 20 mg 20 min						
B P	♂	24			105/57	97/50

Abbreviations as in table III B before A after

Sa_{O_2}	P_{CO_2}	Hb	VC	$FEV_{1\,sec}$	MVV	*Autopsy*	*Biopsy*
98	35	11 9	–	–	–		
93	–	14 1	–	–	–		+
92	–	13 1	–	–	–		+
92	–	13 1	–	–	–		
93	26	12 5	111	84	147	+	
96	25	17 5	–	–	–		
91	37	11 1	74	87	118		
95	34	15 5	–	–	–	+	
96	34	14 7	74	91	129		
84	33	17-	80	100	118		
80	35	16 5	104	79	125		
95	40	14 8	109	84	100		
84	32	10 3	77	80	49	+	
92	36	15 9	102	68	99	+	
93	38	15 6	–	–	–	+	
100	36	14 3	65	90	78		+
79	32	17 3	55	71	52	+	

monary artery wedge pressure mm Hg *CI* cardiac index lit/min/m² *PAR* pulmonary arterial mm Hg Hb hemoglobin g/100 ml VC vital capacity per cent $FEV_{1\,sec}$ forced expiratory

PAW		CI		PAR		SaO_2		PCO_2	
B	A	B	A	B	A	B	A	B	A
						80	70	35	35
8	8	3 4	3 2	284	731	92	90	36	36
–	–	1 7	2 1	–	–	94	100	27	28
5	5	2 4	1 9	1 270	1 665	80	97	21	23
						80	99	35	34
–	–	2 3	2 7	–	–	97	98	27	33
–	–	1 9	2 -	–	–	84	90	32	28
2 5	2 5	2 -	2 9	1 636	932	91	83	28	19
–	–	–	–	–	–	–	–	–	–
–	–	2 -	1 5	–	–	93	86	38	38



Fig 2 Hypertrophy of media and intimal proliferation in small pulmonary artery H E × 240



Fig 3 Intimal proliferation in small pulmonary artery H E, 240

Discussion

Etiology

The following etiological factors have been under discussion in primary pulmonary hypertension

1 Congenital or familial defect in the development of the small pulmonary arteries with persistence of the fetal medial hypertrophy

2 Arteritis of the small pulmonary arteries

3 Repeated small pulmonary emboli from peripheral venous thrombosis

1 The existence of a congenital variety may be supported by the observation of cases presenting symptoms from early childhood or of cases with a family history of primary pulmonary hypertension Three of our patients dated their first symptoms back to the age of 1—5 years In two of these patients there was a family history in that patient R H was a nephew of patient B P Observation of cases with a family history in this condition has also been reported by Clarke et al (2) Dresdale et al (6), Coleman et al (3), van Epps (8) and Melmon and Braunwald (14)

2 Several authors have observed changes of arteritis Rawson and Woske (17) suggest that in at least some cases of pulmonary hypertension the disease may be a form of collagen disease They observed 2 patients with PPH presenting symptoms of Raynaud disease and arthritis and 2 other patients also had a family history of these disorders Taft and Mallory (23) also observed a case of primary pulmonary hypertension with Raynaud s phenomenon Additional cases of proved primary pulmonary hypertension combined with Raynaud s phenomenon have been reported by Wade and Ball 24) Smith and Kroop (19) Celoria et al (1) and Winters et al (28)

3 The repeated occurrence of small pulmonary emboli from peripheral or right heart thrombi has been stressed as the cause of primary pulmonary hypertension especially by Owen et al 16 who observed peripheral thrombosis in 6 and cardiac thrombosis in 2 out of 12 patients with this disorder Similar cases have been observed by Ehrner et al 7 Langfeld et al 12 Heilman et al 10 Goodwin et al 9 Crafoord 4 McGuire et al 13

Rosenberg (18) and Wilhelmsen et al (27) In our material we succeeded in demonstrating peripheral thrombosis in only one of the six patients coming to autopsy In five of the six cases, however, there was a coincidence of organized thrombosis and striking intimal proliferation In three of the cases superimposed fresh thrombi were directly responsible for the sudden fatal outcome Histologically it is very difficult to make a distinction between intimal proliferation and organized thrombosis in the pulmonary arteries The concentric growth and loose cellular connective tissue associated with intimal proliferation may well be interpreted as an organization of thrombotic material with subsequent recanalization of the lumen

From our study we have found support for the following etiological factors in this disease 1 congenital or familial persistence of fetal pulmonary arterioles and 2 repeated small emboli from peripheral venous thrombosis

Treatment

Two methods of treatment have so far been tried in cases of primary pulmonary hypertension

1 Vasodilating agents
2 Anticoagulant treatment

Several agents have been tried in the hope of finding a drug capable of reducing the elevated pulmonary artery pressure selectively without production of systemic arterial hypotension So far no such drug has been found Some agents may reduce the pulmonary artery pressure at least in some patients, but most of these drugs are short acting like Acetylcholine (7, 9, 15) and adrenergic blocking agents Sympatholytic and ganglionic blocking agents also reduce the pulmonary artery pressure (9), but the reduction of the pressure seems to be brought about by its action on the systemic circulation with peripheral pooling of blood and reduced venous return Vasodilating agents such as Theophyl line or Priscoline (6, 15, 30) may produce a fall in pulmonary artery pressure and pulmonary arteriolar resistance Hypotensive agents like Serpasil also seem to be able to bring about a reduction in pulmonary artery presssure, but this agent also acts on the systemic circulation with untoward reactions Our studies with Phentolamine and Vegolysen in one patient confirmed previous studies in that there was a short lasting effect on pulmonary artery pressure

Of the respiratory gases oxygen is well known to bring about a reduction in pulmonary hypertension when this is brought about by anoxemia (20) In obliterative pulmonary hypertension as is found in the disease under discussion, the fall in pulmonary artery pressure is small The problem in these patients is not alveolar hypoventilation On the contrary these patients are hyperventilating as shown by the low PCO_2 found in our study

The concept of repeated small pulmonary emboli from peripheral venous thrombosis as the cause of primary pulmonary hypertension is an attractive one The logical treatment of this condition is anticoagulants This treatment has been tried previously but apart from 2 cases reported by Wood (29) one by Dawson et al (5) and one by Wilcken et

TABLE V Repeated studies of patients on anticoagulants

Name	Sex	Age		BP	PA	PAW	CI	PAR	S_{aO_2}	P_{CO_2}
A.P	♀	32	1959	110/85	125/55	—	3	—	95	34
			1960	110/75	132/60		1 7		94	27
S Å	♀	55	1959	105/95	110/50	5	1 7	1 909	84	33
			1960	115/90	110/45	5	2 4	1 270	80	21
H J	♀	36	1959	120/80	110/42	—	3 1	—	95	40
			1960	140/80	103/46	—	2 3	—	97	27
			1961	150/90	100/40	—	3 4	—	96	35
			1962	155/90	107/42	—	2 9	—	96	27
K H	♀	36	1961	120/80	70/25	5	2 8	597	95	28
			1963	115/90	100/47	3	2 3	1 364	93	26

Abbreviations as in table III

al (26) the treatment has been disappointing as shown by Goodwin et al (9) In order to obtain a distinct effect of anticoagulant treatment in this condition one has to start the treatment at an early stage This is difficult as most patients do not seek medical advice before the disease has lasted for several months or years when dyspnea, cyanosis or syncope make the disease manifest We have tried anticoagulant treatment in several of our cases Ten of our patients have been treated with anticouagulants for periods ranging from 2—5 years The aim of the anticoagulant treatment has been to keep the protrombin proconvertin time in the range of 10—30 per cent This has been achieved at least in the 7 patients who have been controlled by our laboratory Follow up cardiac catheterizations have been carried out in 4 of these patients shown in table V In no patient was there any reduction of pulmonary artery pressure during treatment Six of these patients have died Apparently this treatment was of no success in our cases

In this situation there is a desperate need of a new method of treating these patients The first aim of the treatment must be to reduce the elevated pulmonary arterial pressures, as the pulmonary hypertension apparently is self perpetuating once it is produced It brings about a further rise in pressure by medial hypertrophy of the muscular arteries as a reaction to the hypertension and it may be associated with intimal proliferation and secondary thrombosis The second aim of the treatment must be to create a safety valve for the pulmonary circulation In instances where there is a rise in pulmonary artery pressure, as on exercise it is necessary to shunt off some of the blood to the systemic circulation to reduce the burden placed on an already heavily taxed right ventricle We therefore invented a surgical method of treating pulmonary hypertension by making a "banding"

TABLE VI Blood gas tensions before and after operation on air breathing and 100% O_2 breathing

				Before operation		After operation			
						14 days		6 months	
Name	Sex	Age		Air	100% O_2	Air	100% O_2	Air	100% O_2
I F	♀	23	P_{O_2} mm Hg	65	522	50	87	47	143
			Sa_{O_2} per cent	91		84		82	
			P_{CO_2} mm Hg	36 5	36 5	39	39	38 5	35 5
R F	♂	11	P_{O_2}	68	623	60	595		
			Sa_{O_2}	92		84			
			P_{CO_2}	35	35	35	33		
B E	♀	39	P_{O_2}			60	285	52	526
			Sa_{O_2}	92	—	86		86	
			P_{CO_2}			30	—	28	28
P E	♂	58	P_{O_2}	44	349	Not operated on			
			Sa_{O_2}	77					
			P_{CO}	32	32				

procedure on the pulmonary artery, creating an artificial pulmonic stenosis and secondly by producing a connection between the pulmonic and systemic circulations

In the first patient operated on K H, "banding of the pulmonary artery was carried out, and at the same time an interatrial septal defect was produced This operation was not successful The patient was operated on in hypothermia and tolerated the operation well There was, however, a systemic hypotension following the operation and a tachycardia There was a metabolic (lactic acid) acidosis and pronounced anoxemia, down to 61 per cent arterial O_2 saturation, and the patient died the day after the operation

Later on we abandoned the artificial atrial septal defect and instead created a shunt of the Blalock type making a connection between the subclavian and pulmonary arteries This is not the ideal operation We feel that the best thing to do would be to produce a connection between the aorta and pulmonary artery central to the banding , but owing to technical difficulties we have placed the shunt distal to the artificial pulmonic stenosis So far 3 patients have been operated on with this procedure, patients B E, R F and I F All patients have tolerated the operative procedure well The first days after the operation there has been a distinct cyanosis and the patients have been in respiratory distress We therefore have placed them in a respirator for the first week following the operation During this week the respiratory distress has regressed and the cyanosis has been lessened All patients have recovered and all are feeling better during the observation period, which

TABLE VII Circulatory studies following the 'banding' procedure

	I F		B E.	
Patient	Before	After	Before	After
O_2 satur per cent				
SVC	80	81	62	53
RA	76	74	64	52
RV	76	73	—	47
PA	80	90	65	—
FA	100	92	92	79
Systemic flow lit/min	4 9	5 7	2 9	3 8
Effective pulm flow lit/min	4 9	4 9	2 9	2 3
Shunt right left	0	0 8	0	1 5
Pressures mm Hg				
RA	4	3	7	5
RV	107/0	125/0	—	125/0
PA	107/60	120/78	95/50	—
FA	117/56	126/72	—	—
LA	—	—	—	3

Abbreviations SVC superior vena cava RA right atrium RV right ventricle PA pulmonary artery FA femoral artery LA left atrium

now ranges from 1/2 to 1 1/2 year The improvement has been most pronounced in patient B E who was severely ill at the time of the operation She was in frank right ventricular failure with enlargement of the liver down to five centimeters below the costal margin and peripheral edema Her right ventricular failure has regressed There is no edema and the liver is now no longer palpable She still has dyspnea on exertion, but the dyspnea is less pronounced, and chest pains, which were distressing before operation, have disappeared

The most pronounced change following the operation in these patients is cyanosis We have therefore made blood gas studies in these patients with determination of PO_2 and PCO_2 before and after the operation and at the same time observing the effect of 100 per cent oxygen breathing As shown in table VI in all 3 patients there is a fall in arterial PO_2 and arterial oxygen saturation on air breathing after the operation On 100 % oxygen breathing before the operation one patient, R I , showed a a full rise in PO_2 while the other patient I F demonstrated a small venoarterial pulmonary shunt After the operation there is a pronounced shunt in patient I F and also in patient B E , while patient R I apparently has only a small shunt, his PO_2 on oxygen breathing rising up to 595 mm Hg The varying response to oxygen breathing after the operation apparently is due to the varying size of the shunt produced



Fig 4 Frontal pulmonary angiogram showing site of banding dilated central pulmonary arteries and very narrow or obliterated peripheral pulmonary arteries

It is of interest to note that there has been a rise in arterial PO_2 on oxygen breathing *in patient B E during the* period from 14 days after the operation to 6 months after the operation indicating a decrease in the right to left shunt This finding might indicate that the subclavian pulmonary artery anastomosis has been closed in the intervening period In the same way the high rise in arterial PO_2 on oxygen breathing in patient R F immediately after the operation may indicate that this shunt was already closed at that time

Circulatory studies after the operation have been carried out in patients I F and B E The results are shown in table VII These studies show that there is no fall in pulmonary artery pressure in patient I F and that the pulmonary artery pressure is higher after the operation than before There is also no systolic pressure gradient across the "banding This is apparently due to the left to right shunt exposing the pulmonary artery to systemic arterial pressure The



Fig 5 Pulmonary angiocardiogram showing site of banding and early opacification of aorta through pulmonary artery subclavian artery anastomosis

banding is well seen on the angiocardiograms (figs 4 and 5) The angiocardiograms also demonstrate the subclavian pulmonary artery shunt with early opacification of the aorta The frontal angiocardiogram demonstrates the pruning of the pulmonary arteris (fig 4)

In patient B E cardiac catheterization was carried out from the saphenous vein The catheter passed through a patent foramen ovale into the left atrium and later from the right atrium to the right ventricle but it was impossible to pass the catheter into the pulmonary artery The angiocardiogram in this patient too demonstrated a banding but failed to demonstrate any shunt At the time of the last catheterization the arteri

al PO_2 on oxygen breathing had risen to 526 mm Hg indicating that the shunt had been closed as already mentioned We think that this patient had a patent foramen ovale, and not an atrial septal defect, as no left to-right shunt had been demonstrated on previous catheterizations, nor was it demonstrated during the angiocardiography carried out during the last catheterization The pressure was also higher in the right atrium than in the left during the whole cardiac cycle

Patent foramen ovale is probably the explanation to the low arterial O_2 saturation found in some of these patients (table III) In patient P I arterial O_2 tension was low on air breathing, rising only to 349 mm Hg on oxygen breathing (*table VI*) *He was supposed to have a* patent foramen ovale and this was confirmed at autopsy

The high pressure in the right ventricle following the operation is to be expected We had hoped to demonstrate a pressure fall in the pulmonary artery after the operation as it was found immediately after the banding on the operating table As mentioned there is a high pressure in the pulmonary artery in patient I I where there is a functioning subclavianpulmonary artery anastomosis In patient B F the anastomosis is not functioning and here we might expect a fall in pulmonary artery pressure Unfortunately we were not able to pass the catheter into the pulmonary artery in this patient during the last catheterization

In both patients there has been a rise in cardiac output which might indicate an improvement of the circulation

These studies have shown that the operation carried out has produced a cyanosis and rising cardiac output but no fall in right heart pressures The clinical improvement in these patients, especially patient B I, who was most severely ill at the time of operation with liver enlargement and ankle edema, is a demonstration of the benefit to be gained from this procedure We feel that the main point in the operative procedure is the creation of a safety valve for the pulmonary circulation making it possible to reduce the raised pulmonary artery pressure during a pressure rise on exertion Further follow up studies of these and later patients are, however, needed to make a final judgement of the treatment

As mentioned, superimposed fresh thrombosis of the pulmonary arteries was directly responsible for the fatal outcome in 3 of our patients Anticoagulant treatment in 2 of these patients was unable to prevent the massive thrombosis of the main pulmonary arteries We feel that in these patients pulmonary thrombectomy should be carried out to relieve the obstruction of the pulmonary arteries (Trendelenburg operation) and that banding of the pulmonary artery and subclavianpulmonary artery anastomosis should be carried out at the same time

Summary

The etiology of primary pulmonary hypertension is discussed from the clinical and hemodynamic findings in 17 patients studied by cardiac catheterization Pathological studies have been carried

out in 9 of these cases From this study two etiological factors seem to be important

1 Congenital persistence of fetal pulmonary arterioles and

2 Repeated embolization from peripheral venous thrombosis

In view of the importance of thromboembolism as an etiological factor, 10 of our patients have been treated with anticoagulants for periods ranging from 2 to 5 years This treatment has been disappointing None of the patients has shown any improvement in clinical or hemodynamic findings and 6 of the patients have died while on anticoagulants A new method of treatment has been tried

Banding" of the pulmonary artery, combined with creation of a systemic pulmonary artery shunt Three patients have been operated on with this procedure All patients have improved clinically Hemodynamic studies however have not demonstrated any fall in pulmonary artery pressure A right to left shunt is produced by the procedure with cyanosis of the central type We think that this right to left shunt represents a safety valve for the pulmonary hypertension making it possible to prevent a further rise in pulmonary arterial pressure on exertion

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Conn's Syndrome

A Detailed Study on Corticoid Metabolites in the Urine in Two Operated Cases

By

I Lorenzen, M Damkjær Nielsen and E L Nordentoft

The characteristic disturbance of adreno cortical function in Conn's syndrome is an increased aldosterone production, re sulting in increased urinary excretion of aldosterone However, the aldosterone excretion may vary in primary hyper aldosteronism, and during certain periods it may be within the normal range (5, 6)

The urinary excretion of the other mineralocorticoid metabolites and of glu cocorticoid metabolites is stated to be within the range of normal but in some cases a low glucocorticoid/mineralocor ticoid ratio has been demonstrated (8)

Below follows a report on two cases of primary hyperaldosteronism due to aldos terone producing adrenocortical adeno mas the cases having been followed by repeated analyses of the glucocorticoid and mineralocorticoid metabolites in the urine

Submitted for publication July 19 1965

Methods

The determination of cortisol and corticos terone metabolites was carried out by a modification of the method devised by W S Cost et al (7)

The enzymatic hydrolysis was performed as follows 1/5 of a 24/hour urine was adjusted to pH 4 80 and buffered with sodium acetate buffer to 0 5 N solution β Glucuronidase (2 000 units/ml urine) and sulphatase (1 000 units/ml urine) from the digestive juice of *Helix pomatia* were added and the urine was incubated for 48 hours at 37 C After incubation the urine was extracted with chloroform (3 × 1/3 of the total volume) and re incubated at 37 C for 24 hours with out addition of further enzyme After re in cubation the urine was extracted with 3 × 1/3 the total volume of chloroform and acidified with conc HCl to pH 1 During one hour at room temperature the urine was ex tracted three times with chloroform (each time with 1/3 volume) The combined chlo roform extracts were washed dried and evap orated to dryness in the manner described by Cost

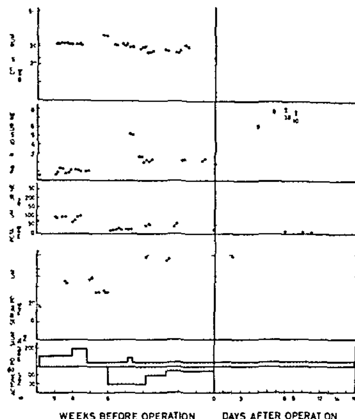


Fig 1 Electrolytes in the serum and urine in case I Effect of Aldactone® potassium and operation (the potassium content in the food is estimated as 50 mEq/day)

The chromatographic fractionations were carried out manually according to the Cost procedure but some of the chromatographic systems for the final purification and semi quantitative determinations were replaced by other systems The system used for separation of the THF allo-THF and THE was toluol methanol water 400 75 25 at 22 C In this system a complete separation of the three steroids was achieved after a 17 hour run For separation of the steroids F and THS the Eberlein Bongiovanni system F B was used at 30 C

Aldosterone excretion was determined by a slight modification of the method of Brodbacher and Hartog 4 The crude urine extract was prepared as described and purified by three chromatographic runs in the following systems

1 Chloroform formamide at 30 C

2 Toluol methanol water 110 70 80 at 22 C

3 Bush C at 30 C

Case reports

Case I A 36-year-old male admitted in the spring of 1963 to Medical Department C for arterial hypertension The hypertension had been diagnosed 5 years previously and had been treated by various antihypertensive agents for the last 4 months before admission exclusively with guanethidine (Ismelin®) Hypokalaemic alkalosis had been diagnosed several times first in 1957

Symptoms on admission fatigue, occasional headache urinary frequency and nycturia There were no pareses paraesthesias tetany or visual disturbances Physical examination revealed no oedema no pareses and no

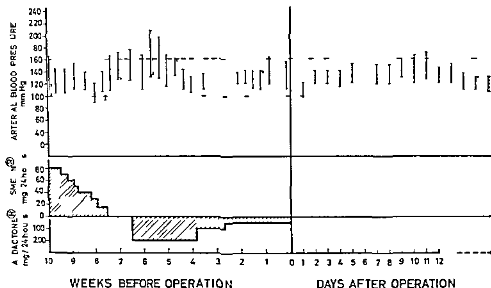


Fig 2 Arterial blood pressure in case 1 before and after the operation The last four values (*) indicate the arterial blood pressure 1 2 4 and 7 months after the operation

tetany The arterial blood pressure was 160/110 (recumbent) and 120/100 (standing) Retinal changes Keith Wagner II Radiography of the heart and lungs showed no abnormality Electrocardiogram isoelectric T waves in the 1st and 2nd standard leads Serum creatinine 1 3 mg/100 ml Periodically the urine contained traces of protein but was normal on microscopy No sugar in the urine The 24 hour output ranged from 1 to 2 litres with a spontaneous concentration ability up to 1 022 The 24 hour excretion of adrenaline and noradrenaline was normal Serum electrolytes normal serum sodium (138 mEq/l) but severe hypokalaemic alkalosis (fig 1) Despite a low serum potassium the 24 hour urinary excretion of potassium was 70 mEq on an ordinary diet The urinary sodium potassium ratio was low The urinary pH was alternately acid and alkaline During the daily administration of 100—200 mEq potassium a total of 1 000 mEq potassium was retained in the course of 3 weeks (fig 1) corresponding to the findings in severe hyperaldosteronism Administration of the aldosterone antagonist Aldactone® (fig 1) entailed an increase in serum potassium a decrease in the urinary excretion of potassium an increase in the sodium excretion and an increase in the urinary ratio of sodium/potassium The arterial hypertension prior to operation may be seen from fig 2

Table I presents the hormone analyses on the urine Before the operation the 24 hour excretion of aldosterone in one of two analyses was definitely increased The excretion of cortisol metabolites was at the upper limit of normal while the excretion of corticosterone metabolites was normal The F/B ratio was elevated on the day before the operation The explanation is possibly the pre-operative state of stress The urine showed prior to operation on paper chromatography of the extracts an extra component in a quantity of 0 8 mg/24 hours This component has not been found in normal subjects and was not present in the urine after the operation

Renal aortography was normal The retroperitoneal insufflation of oxygen gave equivocal information At operation the right adrenal was first exposed and a cystic nodular area making up half the gland was resected Frozen sections showed cortical hyperplasia the zona fasciculata in partic

TABLE I Case 1 K G K (m) Urinary excretion of corticoids mg/24 hr

	2 days preoper	1 day preoper	1 day postoper	2 day postoper	8 months postoper	Normal men (14) Mean values	Normal range (males)
Tetrahydro-F	1 9	2 2	7 2	5 2	1 8	1 2	03 –2 1
Allo-Tetrahydro-F	1 3	1 2	2 0	2 0	1 1	0 7	02 –1 4
Tetrahydro-F	3 1	4 0	6 1	4 2	3 1	2 2	08 –3 2
Reichsteins U	0	0 01	0	0	0 01	0 01	0 –0 03
Cortisol (F)	0 06	0 03	0 10	0 07	0 04	0 04	0 01–0 06
Cortisone (E)	0 03	0 03	0 05	0 04	0 03	0 05	0 02–0 09
Total F	6 39	7 49	15 45	11 51	6 07	4 20	1 3 –6 3
Tetrahydro-S	0 01	0 06	0 65	0 51	0 01	0 03	0 01–0 09
Total 17 OHCS	6 40	7 55	16 10	12 02	6 08	4 23	1 3 –6 3
Tetrahydro-B	0 16	0 06	0 64	0 64	0 12	0 10	0 02–0 19
Allo-Tetrahydro-B	0 30	0 21	1 30	0 77	0 10	0 23	0 06–0 45
Tetrahydro-A	0 10	0 05	0 30	0 08	0 10	0 09	0 05–0 18
Corticosterone (B)	0 02	0 01	0 12	0 04	0 02	0 02	0 –0 04
Comp A	0 01	0 01	0 02	0 01	0	0 01	0 –0 01
Total B	0 59	0 34	2 30	1 54	0 34	0 45	0 13–0 87
Aldosterone μg/24 hr	22	8	0	0	6	8	2–14
Comp \ (UV +BT)	0 8	0 8	0	0	0	0	0
Total F/Total B	11	22	7	8	18	9	5–12

TABLE II Contents of corticoids in adrenal tissue

	Normal tissue[1] (μg g)	Adenoma of case 1 (μg/g)
Aldosterone	0 05 – 0 08	0 47
Corticosterone	0 73 – 2 90	5 32
Cortisol	0 97 – 3 90	9 84

[1] Normal values according to Louis & Conn (9

ular showing signs of activity Exposure of the left adrenal gland revealed a hazel nut-sized adenoma which was enucleated On section the adenoma was of a bright yellow colour Microscopic examination showed cortical adenoma The adenoma weighed 5 g

(The microscopic studies were performed by A Soeborg Ohlsen chief pathologist)

The content of the corticoids aldosterone corticosterone and cortisol in the adenoma was analysed by Ib Brorsen M D (Surgical Department H) This analysis was carried out as advocated by Bojesen (2 3) on a chloroform extract of the tumour tissue The results are given in table II which shows relative to the normal levels an almost tenfold level of aldosterone and doubled levels of corticosterone and cortisol in the tumour tissue

Hormone analyses on the urine after the operation (table I) showed during the first days a high excretion of cortisol and corticosterone metabolites indicating a physiological stress reaction — in accord with which there was during the same period a

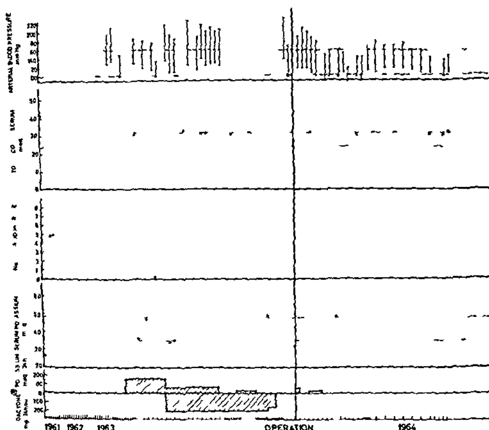


Fig 3 Electrolytes in the serum and urine together with the arterial blood pressure in case 2 Effect of Aldactone® potassium and operation

marked retention of sodium (low urinary sodium/potassium ratio (fig 1)) During the first 2 days after the operation there was no aldosterone in the urine

The serum electrolytes returned to normal soon after the operation Follow up 8 months after the operation showed the serum electrolytes as well as the urinary excretion of hormones including aldosterone to be within the normal range (fig 1 and table I) The arterial hypertension had decreased but had not disappeared Ophthalmoscopy showed normal retinal vessels Electrocardiogram and chest radiography showed no abnormalities.

Case 2 A 46-year-old female in whom benign arterial hypertension had been diagnosed in 1961 During the next 2 years she was treated with various antihypertensive agents Hypokalaemic alkalosis was demonstrated repeatedly but ascribed to chlorothiazide medication In June 1963 the patient was admitted to Medical Department C with fatigue headache and dyspnoea but no complaints of pareses paraesthesiae tetany or visual disturbances At that time she had not received antihypertensive treatment for the past 6 months Physical examination showed no oedema no pareses and no tetany The arterial blood pressure was about 200/120 and there were fundal changes corresponding to Keith Wagner II—III Chest radiography showed increased width of the heart Electrocardiogram showed signs of hypopotassaemia (negative T_1 dipha

TABLE III Case 2 M. H. (f) Urinary excretion of corticoids mg/24 hr

	2 months preoper	9 days postoper	3 months postoper	12 months postoper	Normal females (17) Mean values	Normal range (females)
Tetrahydro-E	0.5	0.2	0.4	0.4	0.9	0.6 – 1.4
Allo-Tetrahydro-F	0.2	0.1	0.2	0.4	0.5	0.2 – 1.5
Tetrahydro-F	0.7	0.1	1.2	0.8	1.9	1.2 – 2.7
Reichsteins U	0	0	0	0.01	0.02	0 – 0.05
Cortisol (F)	0.08	0.11	0.03	0.03	0.04	0.02 – 0.08
Cortisone (E)	0.03	0.03	0.05	0.04	0.06	0.03 – 0.13
Total F	1.51	0.64	1.88	1.68	3.42	2.0 – 5.2
Tetrahydro-S	0.01	0.05	0.04	0.01	0.03	0.01 – 0.09
Total 17 OHCS	1.52	0.69	1.92	1.69	3.45	2.0 – 5.3
Tetrahydro-B	0.16	0.16	0.05	0.07	0.08	0.04 – 0.11
Allo-Tetrahydro B	0.16	0.40	0.11	0.15	0.20	0.08 – 0.36
Tetrahydro-A	0.06	0.01	0.04	0.06	0.03	0.06 – 0.13
Corticosterone (B)	0.03	0.04	0	0	0.01	0 – 0.02
Comp. A	0.05	0.01	0	0	0.01	0 – 0.02
Total B	0.46	0.62	0.20	0.20	0.38	0.12 – 0.57
Aldosterone µg/24 hr				3	8	2 – 14
Total F/Total B	3	1.0	9	6	9	5 – 27

sic T_{III}) and left-sided hypertrophy. Serum creatinine 1.0 mg/100 ml. Periodically the urine contained traces of albumin but no sugar and microscopy was normal. Fig. 3 shows that hypokalaemic alkalosis had been demonstrated repeatedly during the years prior to her admission. Despite a low serum potassium the patient was excreting between 20 and 40 mEq potassium in the 24 hour urine. During the administration of approx. 200 mEq potassium daily for 4 days she retained a total of 484 mEq, a finding suggestive of hyperaldosteronism. The serum sodium was normal or slightly elevated. The urinary sodium potassium ratio (fig. 3) was normal at the time of admission. During administration of potassium the sodium potassium ratio fell, presumably as an indication of an increased aldosterone effect. Aldactone® resulted in an increase in serum potassium and a decrease in total CO_2 in the serum. Urinary hormone analyses prior to the operation may be seen from table III. The excretion of cortisol metabolites was below the normal range, and that of corticosterone metabolites within the normal range, resulting in a low total F/total B ratio. The aldosterone excretion studied once before the operation was within the range of normal. (This aldosterone analysis was carried out by Dr. B. Hökfelt, Karolinska Sjukhuset, Stockholm, Sweden.) Renal aortography showed no abnormality. Retroperitoneal insufflation of oxygen (fig. 4) suggested a tumour in the right adrenal gland.



Fig. 4. Retroperitoneal insufflation of oxygen suggests a tumour of the right adrenal gland

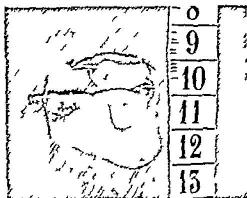


Fig. 5. The cortical adenoma removed together with the right adrenal gland (case 2)

At operation a grape sized tumour (fig. 5) was removed with the entire right adrenal. In this case too the tumour showed the characteristic bright yellow colour on section. Microscopic examination showed cortical adenoma. A biopsy from the right kidney revealed hyperplastic arterioles and a few fibrosed or hyaline glomeruli.

After the operation the serum electrolytes soon returned to normal and the hypertension slowly decreased (fig. 3). Urinary hormone analysis still showed a low F/B ratio two days after the operation (table III) but later an increase to the normal range. One year after the operation electrocardiogram and chest radiography showed no abnormalities. Retinal changes: Keith Wagner II.

Discussion

Both patients exhibited clinical symptoms and signs of hyperaldosteronism and operation revealed in both an adrenocortical adenoma whose removal resulted in normalization of the electrolyte disturbances and a fall in the arterial hypertension thus confirming the clinical diagnosis primary hyperaldosteronism.

The urinary aldosterone analyses demonstrate a fact which was emphasized by Conn (5), viz. that in primary hyperaldosteronism the excretion may be extremely fluctuating in the same patient so that repeated analyses of the urine are needed. Case 1 showed one elevated and one normal aldosterone excretion. In case 2 only one analysis was done showing normal excretion. This may be taken as a sign of hyperaldosteronism in view of the simultaneously low serum potassium (5); repeated investigations might have shown an increased aldosterone excretion.

Determination of cortisol and corticosterone metabolites in the urine of patients with Conn's syndrome has been reported by Cost (8). In two cases he found a low excretion of cortisol metabolites and normal excretion of corticosterone metabolites resulting in a ratio total F/total B low compared with normal subjects. The same finding has been reported by Baulieu et al. (1) and by Pasqualini et al. (10). One of our patients (case 2) had a similar excretion pattern of cortisol and corticosterone metabolites before the operation and

TABLE III. Case 2. M.H. (f) Urinary excretion of corticoids mg/24 hr

	2 months preoper	9 days postoper	3 months postoper	12 months postoper	Normal females (17) Mean values	Normal range (females)
Tetrahydro-F	0.5	0.2	0.4	0.4	0.9	0.6 – 1.4
Allo-Tetrahydro-F	0.2	0.1	0.2	0.4	0.5	0.2 – 1.5
Tetrahydro-E	0.7	0.1	1.2	0.8	1.9	1.2 – 2.7
Reichsteins L	0	0	0	0.01	0.02	0 – 0.05
Cortisol (F)	0.08	0.11	0.03	0.03	0.04	0.02 – 0.08
Cortisone (E)	0.03	0.03	0.05	0.04	0.06	0.03 – 0.13
Total F	1.51	0.64	1.88	1.68	3.42	2.0 – 5.2
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Total 17-OHCS	1.52	0.69	1.92	1.69	3.45	2.0 – 5.3
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Tetrahydro-A	0.06	0.01	0.04	0.06	0.08	0.06 – 0.13
Corticosterone (B)	0.03	0.04	0	0	0.01	0 – 0.02
Comp. A	0.05	0.01	0	0	0.01	0 – 0.02
Total B	0.46	0.62	0.20	0.20	0.38	0.12 – 0.57
Aldosterone µg/24 hr				3	8	2 – 14
Total F/Total B	3	1.0	9	6	9	5 – 27

at T_{11} and left-sided hypertrophy. Serum creatinine 1.0 mg/100 ml. Periodically the urine contained traces of albumin, but no sugar and microscopy was normal. Fig. 3 shows that hypokalaemic alkalosis had been demonstrated repeatedly during the years prior to her admission. Despite a low serum potassium the patient was excreting between 20 and 40 mEq potassium in the 24 hour urine. During the administration of approx. 200 mEq potassium daily for 4 days she retained a total of 484 mEq, a finding suggestive of hyperaldosteronism. The serum sodium was normal or slightly elevated. The urinary sodium/potassium ratio (fig. 3) was normal at the time of admission. During administration of potassium the sodium/potassium ratio fell, presumably as an indication of an increased aldosterone effect. Aldactone® resulted in an increase in serum potassium and a decrease in total CO in the serum. Urinary hormone analyses prior to the operation may be seen from table III. The excretion of cortisol metabolites was below the normal range and that of corticosterone metabolites within the normal range, resulting in a low total F/total B ratio. The aldosterone excretion, studied once before the operation, was within the range of normal. (This aldosterone analysis was carried out by Dr. B. Hokfelt, Karolinska Sjukhuset, Stockholm, Sweden.) Renal aortography showed no abnormality. Retroperitoneal insufflation of oxygen (fig. 4) suggested a tumour in the right adrenal gland.

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Studies on Adipose Tissue from Obese Patients with or without Diabetes Mellitus

I Release of Glycerol and Free Fatty Acids

By

PER BJORNTORP and BERTIL HOOD

In experimental obesity adipose tissue studied in vitro has shown several metabolic abnormalities, viz decreased outflow of free fatty acids (FFA) after epinephrine stimulation and increased lipogenesis from acetate and also from glycerol the glycerol being incorporated into adipose tissue glyceride-glycerol (21) In human obesity abnormalities in the plasma FFA concentration have been described (9, 10 13) Dole (10) reported a fasting level increasing with increasing weight Gordon (13) and Corvilain et al (9) found that in obesity the FFA level was high and did not increase during prolonged fasting as it does in persons of normal weight This was taken as evidence for a decrease in FFA mobilization from adipose tissue, since oxidation of FFA is believed to be unimpaired (14)

Recently evidence was reported that in non diabetic obese patients the elevation of plasma FFA levels after an overnight fast could be lowered by furnishing calories in adequate amount When however, the fast was prolonged and the FFA values for each group were averaged the obese were still found to have higher values One reason for this was thought to be the increased mass of adipose tissue in established obesity (4) Therefore the following study was performed, entailing study of adipose tissue in vitro With respect to lipolytic activity a material of normals was compared with a group of non diabetic obese patients, as well as with a group of obese patients having diabetes mellitus

Submitted for publication November 8 1965

Material

Obesity Six obese women age 17 to 60 years (average 44) weighing 78 to 121 kg (average 94) and all at least 25 % above desirable weight (22) were admitted to the hospital and instructed to eat a 2 500-calorie diet After at least 4 days an intravenous glucose tolerance test (12) was performed which showed a k value of 0 90 to 1 67 (average 1 25) the day before adipose tissue biopsy Fasting blood sugar was 78 to 88 mg per 100 ml blood FFA in venous blood after overnight fasting

914 ± 196 μEq/lit (mean ± standard deviation) None had an infection and all were euthyroid as judged from appearance basal metabolic rate and protein bound iodine These cases were selected as being patients who in the preceding years had gained rapidly in weight and whose history clearly showed overeating or in two cases a maintenance of an earlier caloric intake after a pronounced decrease in physical activity

Diabetic obesity Another six women age 38 to 58 years (average 49) weighed 101 to 140 kg (average 112) all more than 25 % above desirable weight They had constant glucosuria and a fasting blood sugar above 150 mg per 100 ml Mean ± standard deviation of venous FFA was 1340 ± 211 μEq/lit None was on insulin treatment and all were clinically well controlled by moderate restriction of dietary carbohydrates and in some cases by sulfonylurea drugs Drugs were not taken within 24 hours before biopsy

Controls There were thirteen controls consisting of a group of 7 persons operated on for inguinal hernia, 4 men, 3 women, ages 22 to 68 years and a group of other surgical cases 5 with cholelithiasis and 1 with duodenal ulcer 5 women and 1 man (duodenal ulcer) ages 22 to 68 years (average 46) Those in the latter group were under total anesthesia with barbiturates curarin and nitrous oxide when the biopsy was taken while those in the former were anesthetized locally Since no differences could be observed between these two groups in the measured parameters they were treated as one group All had a fasting blood sugar of less than 90 mg per 100 ml no glucosuria and were in caloric balance as judged from their histories Glucose tolerance tests performed in 6 of these cases gave k values of 1 40 to 2 19 None had an infection nor had any severe disease except as mentioned They were all within ± 10 % of desirable weight (22)

Methods

After an overnight fast an infiltration anesthesia was performed intradermally on the abdominal wall in a rhomboid figure in the lower, lateral quadrant After an incision in the middle of the rhomboid about 5 g of adipose tissue was carefully removed cut by scissors into several smaller pieces of about 25—50 mg and immersed in 10 ml of 4 % albumin in Krebs Ringer bicarbonate buffer of pH 7 4, containing 10 mM glucose This solution was at room temperature The pieces of adipose tissue were when necessary cut into smaller pieces weighing about 25 mg each About 200 mg of adipose tissue was quickly weighed on a torsion balance and incubated within 10 minutes after removal

The incubation flasks were 50 ml cylindrical, flat bottom tubes (Hagedorn tubes) which were siliconized The basal incubation medium was 3 ml 4 % albumin in Krebs Ringer bicarbonate buffer, pH 7 4 containing 10 mM glucose Ten flasks were incubated The first four contained no additions another two flasks contained 8 μg/ml of norepinephrine and two flasks 10 000 micro units of insulin Finally two flasks served as controls without adipose tissue After pre incubation for 30 minutes the tissue in two of the flasks without a hormone supplement was analyzed for fatty acids as described below The flasks were then left in a Dubnoff type incubator for 2 hours Temperature was 37 and oscillations 60 per minute Samples of media were taken at 0 and 120 minutes of incubation for FFA analyses by a modified colorimetric method (2, 11) Glycerol was estimated by periodate oxidation (18)

After incubation the adipose tissue was removed from the flasks, rinsed briefly in Krebs Ringer bicarbonate buffer and then extracted with 15 ml of chloroform methanol as previously described (5) Fatty acids were then determined in the chloroform phase (2 11) Methods for determination of triglyceride (8) lipid phosphorus (24) and DNA (28) in the tissue and for histological determination of the number of cells have been described earlier (5)

The albumin used was bovine serum albumin (Armour Fraction V Batch HG 1371) Norepinephrine was from Astra and insulin (crystalline bovine plus pig insulin) from Nordisk Insulin

TABLE I Glycerol release and increase of total free fatty acid pool of medium and tissue after incubation of human subcutaneous adipose tissue in vitro. Mean ± SEM

	Glycerol release		FFA increase[1]	
	(mμmoles/μgDNA/h)	(mμmoles/10^4 cells/h)	(mμmoles/μgDNA/h)	(mμmoles/10^4 cells/h)
Controls	7.1 ± 1.9	4.9 ± 2.4	2.1 ± 1.1	2.0 ± 2.4
Obesity	8.8 ± 3.2	8.1 ± 2.6	4.8 ± 1.9	5.8 ± 2.4
Diabetic obesity	15.6 ± 3.8	12.5 ± 2.6	21.4 ± 5.2	24.7 ± 6.0

[1] Determined as amount of FFA in medium at 120 minutes minus that at 0 minutes plus amount of FFA per unit adipose tissue at 120 minutes minus that at 0 minutes

TABLE II Glycerol release and increase of total free fatty acid pool of medium and tissue after incubation of human subcutaneous adipose tissue in vitro in the presence of 8 μg/ml norepinephrine

	Glycerol release		FFA increase[1]	
	(mμmoles/μg DNA/h)	(mμmoles/10^4 cells/h)	(mμmoles/μgDNA/h)	(mμmoles/10^4 cells/h)
Controls	10.5 ± 2.6	7.3 ± 2.8	14.3 ± 2.1	10.4 ± 3.0
Obesity	12.4 ± 4.7	10.3 ± 3.1	11.2 ± 3.0	13.1 ± 3.4
Diabetic obesity	15.0 ± 6.0	12.3 ± 2.9	35.6 ± 5.0	32.0 ± 4.8

[1] Calculated as in table I

Results

The results of determinations of fatty acids and glycerol in the basal state of the tissue (not stimulated by norepinephrine or insulin) are listed in table I. Units of DNA and number of fat cells have both been used for reference purposes (5). It may be seen that there were no significant differences between controls and obese persons in any of the parameters measured. Diabetic obese patients, however, showed a significantly higher increase of the total fatty acid pool ($p < 0.02$) as well as an increase of glycerol release ($p < 0.05$) as compared with controls.

Table II gives the corresponding values after stimulation with norepinephrine. Glycerol release of controls and obese now had increased to levels where no differences between groups longer were found. When fatty acid pool figures are compared, however, there was a significant difference between diabetic obesity and the other groups ($p < 0.002$ as compared with controls, $p < 0.01$ as compared with obesity) with considerably higher values in the former.

After stimulation with insulin the results given in table III were obtained

pose tissue (17), described in the rat (3, 23, 26) and in the human (1, 15)

The non-diabetic obese group showed no differences from the controls, except for the pronounced response of fatty acid release to insulin This holds even for the response to a large amount of norepinephrine where the mean increases in glycerol release over control values were nearly identical The present study thus furnishes no support for an abnormality in adipose tissue of obese patients as an explanation for the increase in plasma free fatty acids after prolonged fasting also occurring after a standardized diet (4) The present results together with previously reported results on the number of fat cells in obesity (7) rather seem to indicate that the mentioned increase is caused by an increased number of normally functioning adipose tissue cells

The pronounced contraction of the adipose tissue pool in obesity after insulin addition is difficult to evaluate because of the large amounts of insulin added It has to be kept in mind, however that one explanation might be an increased sensitivity of adipose tissue to insulin in the obese

Summary

Subcutaneous adipose tissue from obese diabetic obese and control patients was incubated *in vitro under basal conditions* and after addition of large amounts of either norepinephrine or insulin Basal glycerol release was increased in the diabetic group, as was also the total fatty acid pool After addition of epinephrine a high glycerol release was found and the differences had disappeared, while the fatty acid pool size of the diabetic tissues was still elevated above that of the other groups After addition of large amounts of insulin, glycerol release was equal in all groups The fatty acid pool had decreased in all groups to very low values and no differences were found The obese group showed no differences as compared with the controls except for a more pronounced contraction of the free fatty acid pool after addition of insulin The results are compatible with a concept of increased lipolysis and decreased esterification — both possibly indications of a deficient insulin effect on adipose tissue — in the obese diabetic group, and of normally functioning lipolytic and fatty acid re esterification systems in adipose tissue of the obese group without diabetes mellitus

Acknowledgements

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Studies on Adipose Tissue from Obese Patients with or without Diabetes Mellitus

II Basal and Insulin stimulated Glucose Metabolism

By

PER BJORNTORP

It has recently been reported that adipose tissue in vitro from obese patients with diabetes mellitus released more glycerol and fatty acids per fat cell than controls (4) The high release of fatty acids was probably caused not only by a decreased re esterification but also by an increased lipolytic activity Both these defects could be corrected by addition of large amounts of insulin and were therefore suggested to be caused by a deficiency in adipose tissue with respect to insulin level or insulin sensitivity (4)

Östman (13) recently found that adipose tissue from young insulin dependent diabetic patients showed a decreased re esterification of fatty acids as measured by incorporation of labelled fatty acids into triglycerides in vitro and probably also an increased lipolysis while glucose uptake was not diminished. No differences were found between adult diabetics and controls

In the present work adipose tissue from obese patients with and without diabetes mellitus has been compared with tissue from controls with respect to insulin effects on certain metabolic processes centered on glucose uptake Total glucose uptake and the incorporation of 1 ^{14}C-glucose into carbon dioxide and lipid were selected for investigation, these incorporations being increased by insulin in rat adipose tissue (8)

Material and methods

The material comprised 6 non-diabetic obese patients all more than 25 % above desirable weight (12) and all with a k value above 0.90 for the intravenous glucose tolerance test Six diabetic obese patients were also more than 25 % overweight, had constant glucosuria and fasting blood sugar above 150 mg per 100 ml Good control of their diabetes mellitus was obtained by dietary means and/or sulfonylurea drugs The controls were 13 patients operated on for hernia and patients with gall-stone or duodenal ulcer The material has previously been described in more detail (4)

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TABLE I Glucose uptake by human subcutaneous adipose tissue in vitro Mean ± SEM

	mg/mg DNA/h		mg/10⁷ cells/h	
	No addition	Insulin (10 000 μU/ml)	No addition	Insulin (10 000 μU/ml)
Controls	2 1±0 5	2 1±0 6	1 7±0 3	1 9±0 4
Obesity	2 9±0 7	3 3±0 4	3 7±0 9	3 8±0 4
Diabetic obesity	4 1±0 6	3 9±0 8	3 5±0 5	4 5±0 9
Controls Obesity	p > 0 10	p > 0 10	p < 0 05	p < 0 01
Controls Diabetic obesity	p < 0 05	p < 0 10 > 0 05	p < 0 01	p < 0 05
Obesity Diabetic obesity	p > 0 10	p > 0 10	p > 0 10	p > 0 10

Adipose tissue from the subcutaneous stores lateral to the umbilicus was excised, dissected into 25—40 mg pieces and incubated in a Krebs Ringer bicarbonate medium containing albumin and glucose at final concentrations of 4 % and 10 mM respectively as previously described (4) 1 ¹⁴C glucose (The Radiochemical Centre, Amersham CFA 204) was added in an amount corresponding to about 250 000 cpm Incubations were performed in cylindrical glass tubes, sealed with a rubber stopper pierced by two glass tubes closed with rubber membranes as earlier described (2) Six tubes were incubated in each experiment The first two contained no additions to the basal medium *to another two was added 10 000 microunits/* ml of insulin, and two further tubes were incubated without tissue as a control Enzymatic activities were stopped after 150 minutes of incubation by injecting 0 2 ml N sulfuric acid through the rubber membrane Radioactivity in carbon dioxide was then collected and counted, and radioactivity in lipid extracted and counted as described elsewhere (2) Glucose was determined enzymatically (10) in the incubation medium at 0 and 150 minutes of incubation

Results of radioactive experiments were expressed as glucose converted to carbon dioxide or lipid calculated from counts found in these products and from the values for the original specific activity of the medium

The insulin used was mixed crystalline bovine and pig insulin (Nordisk Insulin)

Results

Table I gives the results of the glucose-uptake measurements expressed on the basis both of DNA and of the number of adipose tissue fat cells (5) In the basal state the tissue from obese patients with or without diabetes took up more glucose than the controls, on a DNA basis the difference was statistically significant only for the diabetic obese group The obese groups were not different from each other After insulin addition, differences between the both obese groups and the controls could again be found on a cell number basis while on a DNA basis the difference was found only as a trend for the diabetic obese group Insulin did not increase glucose uptake in any of the groups

In table II the values for incorporation of label from 1-¹⁴C glucose into carbon dioxide are listed in the basal state and after stimulation with insulin The non diabetic obese group incorporated more label into carbon dioxide than the controls or the diabetic obese group Between the latter two groups no differences could be demonstrated After stimulation with insulin, carbon dioxide

TABLE II Conversion of 1 ¹⁴C-glucose into carbon dioxide in human subcutaneous adipose tissue in vitro Mean ± SEM

	Millimicromoles/μg DNA/h		Millimicromoles/10⁴ cells/h	
	No addition	Insulin (10 000 μU/ml)	No addition	Insulin (10 000 μU/ml)
Controls	0 17±0 02	0 31±[1]0 05	0 14±0 02	0 25±[1]0 04
Obesity	0 25±0 03	0 45±[1]0 07	0 39±0 05	0 58±[2]0 09
Diabetic obesity	0 19±0 03	0 26± 0 04	0 18±0 03	0 25± 0 04
Controls-Obesity	p < 0 05	p > 0 10	p < 0 001	p < 0 01
Controls-Diabetic obesity	p > 0 10	p > 0 10	p > 0 10	p > 0 10
Obesity Diabetic obesity	p < 0 10 > 0 05	p < 0 05	p < 0 05	p < 0 01

[1] p < 0 05 increase as compared with no addition
[2] p < 0 10 > 0 05 increase as compared with no addition

TABLE III Conversion of 1 ¹⁴C-glucose into lipids in human subcutaneous adipose tissue in vitro Mean ± SEM

	Millimicromoles/μg DNA/h		Millimicromoles/10⁴ cells/h	
	No addition	Insulin (10 000 μU/ml)	No addition	Insulin (10 000 μU/ml)
Controls	0 75±0 14	0 64±0 09	0 59±0 11	0 54±0 08
Obesity	0 86±0 13	0 70±0 12	1 24±0 19	1 38±0 24
Diabetic obesity	0 60±0 13	0 54±0 08	0 63±0 14	0 56±0 08
Controls Obesity	p > 0 10	p > 0 10	p < 0 02	p < 0 01
Controls-Diabetic obesity	p > 0 10	p > 0 10	p > 0 10	p > 0 10
Obesity Diabetic obesity	p > 0 10	p > 0 10	p < 0 05	p < 0 01

incorporation was again higher in the non diabetic obese group as compared with the controls and the diabetic obese group on a cell number basis, but on a DNA basis a difference was statistically demonstrable only in the comparison between the obese groups Insulin increased incorporation of labelled glucose into carbon dioxide in controls and non diabetic obesity but not in diabetic obesity

The results of incorporation of label from 1 ¹⁴C glucose into lipids are given in table III The non-diabetic obese group incorporated more label into lipid than the controls and the diabetic obese group on a cell number basis but not on a DNA basis No difference was

found between controls and diabetic obesity After addition of insulin the same results were found Insulin did not increase incorporation into lipid in any group

Discussion

Since the cell number basis and the DNA basis are not exactly equivalent as reference units for adipose tissue cell activities (5), both have been used in the present work Generally good agreement between results utilizing these reference bases was found, but in some instances differences found between means were statistically significant only on cell number basis

The biopsies of adipose tissue used in the present work show a relatively small response to hormones as far as the reactions measured here are concerned Thus only a moderate increase of incorporation of label from 1-^{14}C glucose into carbon dioxide and lipid occurred with large amounts of norepinephrine (3) and with large amounts of insulin there was only an increase of incorporation of label into carbon dioxide With other types of biopsies, it is possible to get more pronounced hormone stimulation (6, 11) but in order to compare the results obtained here with those previously described for fatty acid release and lipolysis of adipose tissue in vitro from the patient groups in question (4), the present type biopsy was used

Glucose uptake was increased in both the obese groups as compared with the controls This increase was parallelled by an increase in incorporation of label from 1-^{14}C glucose into both lipid and carbon dioxide in the obese group without diabetic symptoms The diabetic group, however, showed a different metabolic pattern Here the increased glucose uptake could not be explained by an increase in label incorporated from 1-^{14}C glucose into carbon dioxide or lipid, and has to be explained by an increase in other metabolites not identified

Insulin added to rat adipose tissue in vitro produces an increased transfer of label from 1-^{14}C-glucose to carbon dioxide and lipid (8) This is the pattern found in the present work in the obese non diabetic group, and might thus imply an increased influence of insulin on adipose tissue in vivo demonstrable in vitro This finding, as well as previously reported increased levels of immunologically determinable plasma insulin in obesity (9), thus suggests an increased influence of insulin on adipose tissue in obesity without diabetes mellitus

It seems however puzzling that insulin added in vitro in large amounts, far above those expected to act on adipose tissue in vivo, increased incorporation into carbon dioxide and lipid only to a limited degree or not at all This finding is in accordance with reports by Martinsson (11) and Hirsch and Goldrick (6), who also found a limited in vitro insulin response for certain preparations of adipose tissue Among other factors the trauma during preparation might be responsible for the damage to the delicate insulin response mechanism of the tissue, in accordance with results in rat epididymal fat pads (1) However in respect of the only parameter where insulin re

sponse is measurable in the preparations used in the present work viz incorporation of 1-^{14}C glucose into carbon dioxide, no measurable increase was found in the diabetic group, while such a response was found in both non diabetic groups The technical disadvantages of the biopsy used, as mentioned, together with the large amounts of insulin added, seem to make it difficult to argue from the present in vitro system to the possible situation in vivo The findings might indicate that the insulin effect on the metabolism of glucose in the fat cell in human diabetes mellitus is diminished This is now further investigated under more physiological conditions, with smaller insulin additions, to systems with optimal conditions for insulin response in vitro as worked out by Bjorntorp and Martinsson (6)

Summary

Human subcutaneous adipose tissue from control, obese and diabetic obese patients was incubated in vitro, and glucose uptake and the incorporation of label from 1-^{14}C glucose into carbon dioxide and lipid were measured in the basal state and after insulin addition Glucose uptake per adipose tissue cell was increased in both the obese groups as compared with the controls In non diabetic obesity this corresponded to an increase of incorporation of label into the products mentioned a result compatible with an enhanced insulin effect on adipose tissue The diabetic tissues however showed no increase in these incorporations nor was there an increase in incorporation of 1-^{14}C glucose into carbon dioxide after insulin stimulation as found for the non diabetic groups

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Frequency of Pathological Proteins (M-components) in 6,995 Sera from an Adult Population

By

U Axelsson, R Bachmann and J Hällen

Pathological proteins (34) or M components (25) in the serum were formerly observed mainly in myelomatosis and macroglobulinaemia Waldenstrom In recent years such components have been found with increasing frequency also in patients with other diseases (1, 4, 9, 17, 19, 20, 22, 26 29, 30 31, 32) and even in apparently healthy persons (10 14) Knowledge of the frequency of M components in a random population would help us to understand the significance of the occurrence of such proteins

A paper electrophoretic analysis of sera from 294 largely healthy subjects above 70 years was described in a previous publication (10) In none of the 9 subjects with M components did subsequent investigation warrant a diagnosis of myelomatosis or macroglobulinaemia Waldenstrom with certainty Several of these subjects have since died from cancer of various organs

A mass health control of the population of the district of Varmland in Sweden offered an opportunity to study the frequency of pathological proteins in a larger series covering a wider age range

The subjects in whom paper electrophoresis revealed M components were studied clinically, roentgenologically, cytologically and immunologically

Material and design of investigation

Sera were collected during a 3 month period (April June 1964) in 4 parishes (Ekshärad Gustaf Adolf Rammen and Norra Råda) All persons above 25 years and living in this area were invited to take part in the mass health control 79 % accepted Blood samples were collected from the 7 918 persons On account of the fact that sera could not be sent to us every day samples from 6 995 persons were placed at our disposal These samples which covered 70 % of the population above 25 years were on the whole just as representative as the entire number of samples from 79 % of the population The 6 995 sera derived from 3 400 men with a mean age of 51 3 years and 3 595 women with a mean age of 50 3 years The age and sex distribution as well as the percentages of persons who

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cooperated in the various age classes are given in table I

The blood samples were collected between 1 p m and 8 p m During transport to Malmo, which lasted about 10 hours, the sera were kept at + 5° — + 10 C, after which they were stored at — 20 C until analysed

All sera were studied by paper electrophoresis The strips were inspected visually for M components by two independent examiners M components were said to be present when a discrete, narrow extra fraction was seen in the paper electrophoretic strip Sometimes an increased concentration of a normally occurring fraction suggested the possible occurrence of an M component of equal electrophoretic mobility An M component was then said to be present if immunoelectrophoresis revealed a corresponding bow in any of the precipitation lines of the immunoglobulins

When M components were demonstrated the paper electrophoretic fractions were studied quantitatively

The M components were also classified immunologically

Persons with M components were requested to present themselves for medical examination (during July—September 1964) by one (U A) of us, who was stationed in Varmland Examination included a detailed history of the patient general physical examination ophthalmoscopy and sternal puncture The laboratory studies included determination of the haemoglobin (Hb) white blood cell count (W B C) differential count, erythrocyte sedimentation rate (E S R — Westergren) and examination of the urine for protein In cases with proteinuria paper electrophoresis was done on concentrated urine

Two marrow smears from each person were examined The number of plasma cells and lymphocytes per 500 nucleated cells was counted in each smear The preparations were closely examined for mast cells and a large number of plasma cells were studied for atypical features and properties known to be common in the presence of myelomatosis with M components of γA type (23) As in the investigation by Drivsholm and Clausen (6) a distinction was made between blue grey hyaline intranuclear inclusions surrounded by a dark rim and others Marrow smears from 15 supposedly healthy subjects in Gormsen s series (8) were examined for plasma cells with inclusions flaming and compartments

The examination also included roentgenography of the skeleton For technical reasons the examination included only the skull in 10 cases, but otherwise also the thoracic and lumbar spine, pelvis proximal parts of the upper arms and the thighs

Methods

Paper electrophoresis was done according to the method of Laurell et al (13) With this method the distance between the β_1 globulin fraction and the most cáthodal part of the γ globulin fraction is 5—6 cm The concentration of the M components was determined either by elution of the M component separately and subsequent subtraction of the estimated concentration of the normal serum proteins with the same paper electrophoretic mobility or by visual evaluation of the concentration of the M component compared with that of other protein fractions particularly of the β_1 globulins The latter method was used mainly for estimation of the concentration of M components in low concentration (< 0 5 g/100 ml)

The serum protein concentration was determined by a biuret method (24)

Immunological classification of the M components was done by the methods used by Bachmann and Laurell (2) and *zinc turbidimetric determination* according to Kunkel (11)

Heller s test with nitric acid and heated urine and water in equal parts (sensitivity down to about 6 mg/100 ml) was used for demonstrating proteinuria

Concentration of urine was done with the aid of a collodium tube (Membranfiltergesellschaft Gottingen Germany) according to Mies (15)

Bone marrow smears were stained according to May Grunwald Giemsa at pH 7 0

TABLE 1 Proportion of population above 25 years examined and incidence of M-components in male and female grouped according to age

	Subjects studied				M-components found					
	Male		Female		Male		Female		Total	
Age	No	%[1]	No	%[1]	No	%[2]	No	%[2]	No	%[2]
25–29	208	58	287	76	0	0	0	0	0	0
30–39	617	70	664	83	0	0	3	0 5	3	0 2
40–49	762	70	783	83	1	0 1	1	0 1	2	0 1
50–59	772	71	859	80	8	1 0	10	1 2	18	1 1
60–69	662	61	634	69	13	2 0	9	1 4	22	1 7
70–79	296	56	315	55	7	2 4	5	1 6	12	2 0
80–89	76	43	48	31	7	9 2	0	0	7	5 7
90–99	7	33	5	14	0	0	0	0	0	0
Total	3 400	66	3 595	74	36	1 1	28	0 8	64	0 9

[1] Per cent of total population in each age group
[2] Per cent of subjects examined in each age group

Results

Frequency of M components age- and sex distribution

M-components were suspected or demonstrated in 73 of the 6,995 sera M-components satisfying the above mentioned criteria were demonstrated in 64 *sera corresponding to a frequency* of 0 9 % These sera were from 36 males (mean age 68 years) and 28 females (mean age 59 years) The mean age in this group was 64 years (table I) The incidence of the M components increased significantly ($P < 0\ 001$) *with* age and was highest (5 7 %) in the age class 80—89 years The increase appeared more marked for the men than for the women, bu this difference was statistically significant only for the 80—89 year age class ($P = 0\ 042$)

Paper electrophoresis of the 64 sera with M components

The mobility of the M components varied between that of β_1 and that of γ_2 Their concentrations are given in fig 1 (for sera with two M components *the values given indicate the sum of the* concentrations of both) In most (84 %) of the sera the concentration of the M-components was $\leqslant 1\ 0$ g/100 ml The highest was 2 4 g/100 ml

The concentration of the paper electro*phoretic gammaglobulin fraction* (excluding M-components with the same electrophoretic mobility) is given in fig 2, together with the concentration of the M components In most of the sera the values crowded around the lower border of the normal range In 15 sera

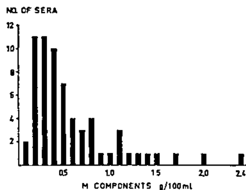


Fig 1 Concentration of M components in 64 sera

the concentration was clearly low ($\leqslant$ 0 5 g/100 ml) In no instance was it clearly increased ($>$ 1 3 g/100 ml)

The albumin concentration was never decreased ($<$ 4 2 g/100 ml) The α_2-fraction was increased ($>$ 0 66 g/100 ml) in 5 sera

Immunological classification In 39 sera (61 %) the M components were of type γG, in 17 (27 %) of type γA and in 5 (8 %) of type γM Three (5 %) sera contained 2 components, namely γG + γM in 2 and γA + γG in 1 In the former 2 the γG M component was the larger fraction and in the latter case the γA M component

Paper electrophoresis of the urine was done in the 5 cases in which proteinuria was demonstrated No M component of the type light chain protein could be detected

E S R In fig 3 the E S R is presented together with the concentration of the M components In 11 (18 %) cases the E S R was normal (males $<$ 8, females $<$ 12 mm/1 hour) and in 27 (43 %), including 2 with increased α_2 fraction, it was over 20 mm/1 hour In several cases with M components in relatively high concentration the E S R was only slightly raised

Zinc turbidimetric determination The values were normal in 39 cases (γG 30 γA 6, γM 2, γG + γM 1 case), decreased in 19 (γG 5, γA 11, γM 2, γA + γG 1 case) and increased in 6 (γG 4, γM 2, γG + γM 1 case)

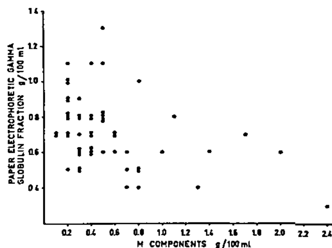


Fig 2 Paper electrophoretic gammaglobulin fraction (excluding any M components of same mobility) and M components in 64 sera

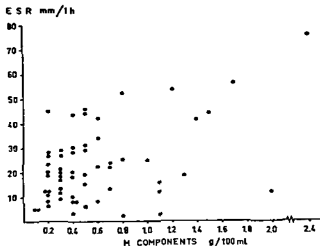


Fig 3 E S R and concentration of M-components in 62 subjects

Bone marrow Sternal puncture was done in 63 cases (one refused) In one case the yield was very poor, and no differential count was made In 6 cases the number of bone marrow cells in the smears was somewhat low but in the other cases the yield of the puncture was good

The mean number of lymphocytes (excluding one case of lymphatic leukaemia) was 15 9 %, standard deviation 4 9 % and the error of the method per 1,000 counted cells 1 3 % The corresponding figures for plasma cells were 2 7 % 3 4 % and 0 4 %

Fig 4 shows that 23 % of the cases had more than 3 % plasma cells Nine had between 3 % and 5 % and 6 had 6 % 6 % 7 % 9 %, 18 % 19 %

In the 2 smears with the largest number of plasma cells (cases 6884 and 7006) the nuclei and the cytoplasm varied in form and size and here and there plasmocytic reticulum cells (27) were seen In one smear with 2 % plasma cells of homogenous appearance, some were seen in clusters which did not happen to be included in the differential count

Of 1 500 plasma cells in bone marrow smears from 15 supposedly healthy persons a pink, intranuclear inclusion was seen in one flaming in none, and compartments in 8, including 2 discovered among 100 plasma cells in one and

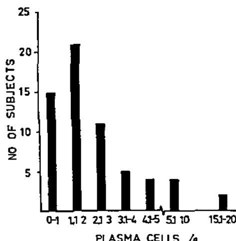


Fig 4 Plasma cells in bone marrow in 62 subjects with M-components

TABLE II Subjects with special types of bone marrow plasma cells

Type of plasma cell	*Of 18 subjects with* γA M components	*Of 35 subjects with* γG M components
1	2	3
2	2	0
3	2	0
4	5	0

Type 1 with blue grey hyaline intranuclear inclusion body surrounded by a dark rim Type 2 with other types of intranuclear inclusion bodies Type 3 with flaming Type 4 with compartments

the same smear A hundred plasma cells were studied for these properties in each case of our series with M components except in 4 cases in which the cell density of the smear and the percentage of plasma cells were too low These 4 cases are not included in what follows Table II shows the results of this part of the investigation In view of the findings in the controls, only cases with more than 2 cells with compartments or one inclusion body per 100 cells will be accounted for The number of cells with blue grey inclusion bodies with surrounding rims was 2/100 and 33/100 in 2 cases in the γA group and 2/100, 10/100 and 20/100 and 3 cases in the γG group The number of cells with inclusion bodies of the other type was 2/100 and 4/100 the number of flaming cells was 3/100 and 9/100 and cells with compartments 3,3,4,9 and 15/100, all these cells were found on counting 100 plasma cells in smears from the γA group In several cases more than 100 plasma cells were seen and then in 2 preparations from cases with γ G M component one respectively two flaming cells were observed The incidence of these features did not vary with the number of plasma cells or the concentration of the M component, 4 cells with compartments and one with a pale inclusion body were seen, for example, in one case with 1 % plasma cells and an M component (γA) in a concentration of 0 2 g/100 ml, while in 2 cases with 19 % and 18 % plasma cells and M components (γA) in concentrations of 1 0 respectively 2 4 g/100 ml the plasma cells showed nothing remarkable apart from marked polymorphism

In one case (apparently healthy 50 year old woman with 4 % plasma cells, M component (γA) in a concentration of 1 6 g/100 ml, E S R now 56 mm, and E S R increase to 20—30 mm known for about 10 years) 95 % of the plasma cells showed vacuolated cytoplasm, which in some cells was divided into compartments In several cells the cytoplasm showed patches of eosinophilic material especially in the vacuoles (fig 5, case 7848)

In 7 cases with M components of type γM, the number of plasma cells was not more than 1 5 % except in one

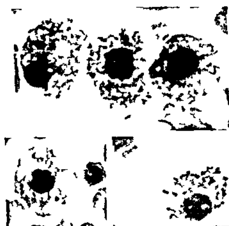


Fig 5 Case 7848 vacuolated plasma cells with eosinophilic patches and compartments



Fig 6 Case 7677 two lymphoid cells low right

case with 4 %, and showed nothing morphologically remarkable except in the last mentioned case where most of them were small lymphoid cells Apart from a man with lymphatic leukaemia and γG M component the largest relative number of lymphocytes in the marrow namely 29 %, was seen in a 76-year old woman (case 7677) with γM M component (0 2 g/100 ml) Of these lymphocytes several had irregularly shaped nuclei with loose chromatin and nucleoli like areas and irregularly out lined cytoplasm (fig 6) This woman who had a long history of moderate anaemia felt well the peripheral blood contained 26 % normal lymphocytes (W B C 7,000/mm³) and the marrow showed an increased number of mast cells (3—4 per smear) Such a number of mast cells were seen also in 3 other cases with M components of type γA γG, γG + γM in the last two mentioned however the preparations were very rich in cells

Roentgenography One woman (Case 8420) had myeloma like changes in the skull (see case reports) Two women (75 and 60 years) had slight and one woman (76 years) marked osteoporosis In the latter case the concentration of the M component was 0 5 g/100 ml and the number of plasma cells in the marrow was 4 % Apart from degenerative joint lesions nothing remarkable was seen in the series except an obscure change in the cavitas glenoidalis scapula dxt in one case The nature of this change could not be cleared up It was seen in an apparently healthy woman with moderate brachialgia, the serum electrophoretic pattern was normal apart from an M component of 0 2 g/100 ml, the E S R 6 mm and the Hb was 14 2 g/100 ml

State of health Fifty nine of the 64 persons presented themselves for medical examination Three had been admitted to hospitals from which we obtained information, and one had emigrated to U S A and was examined there A feeble minded woman (6359) did not come for examination but information about her was obtained from the records of a hospital where she had been admitted in February 1965 because

TABLE III Data concerning the 7 cases described in detail

Case	Sex	Age	Hb (g/100 ml)	W B C	Bone marrow plasma cells (%)	E S R	Gammaglobulin[1] (g/100 ml)	M-component (g/100 ml)	M-component (type)	Skeletal X ray appearance
6140	M	61	13 3	7 600	3	19	0 4	1 3	γG	Normal
8416	M	56	16 5	15 200	9	19	0 9	0 5	γA	Normal
6497	M	75	16 0	7 200	7	55	0 6	0 4	γG	Normal
6350	M	87	13 6	20 500	1	12	0 3	1 1	γG	Normal
6844	M	73	12 4	6 300	18	76	0 3	2 4	γA	Normal
7006	M	67	14 7	10 000	19	25	0 6	1 0	γA	Normal
8420	F	76	13 9	11 900	4	54	0 8	0 9+0 3	γA+γG	Abnormal

[1] Paper electrophoretic gammaglobulin fraction excluding any M components of same mobility

of an attack of hypoglycaemia following medication with chlorpropamide

Analysis of the history, general physical examination or laboratory studies revealed nothing remarkable in 21, who were classified as healthy Abnormalities were found in 36, but in only few of these cases was their normal activity impaired To this group belonged 22 persons with cardiovascular and/or cerebrovascular diseases including 8 with substantial symptoms In addition some subjects had diabetes, gallbladder symptoms, peptic ulcer pyelonephritis, spondylosis, severe psychasthenia, intestinal cancer (2 healthy men had been operated upon 6 and 8 years previously), severe infection with actinomyces 18 years previously, and a well controlled pernicious anaemia Anaemia (Hb < 12 g/100 ml) was noted in 3 women Two had 11 8 g/100 ml, one of them had had a low value for several years One, who had 9 8 g/100 ml, was studied further and found to have sideropenia *but no demonstrable source of bleeding* In an apparently healthy 78 year old man the Hb was 11 5 g/100 ml Two persons had unexplained leucocytosis (16 000 and 14,000) with normal differential count respectively 77 % neutrophilic granulocytes

In 7 cases presented below more noteworthy findings were made Data on them are given in table III

Case 6140 (A S) A 61 year old man, whose father had died from prostatic cancer and whose brother had died from leukaemia For the last 20 years he had bruised readily In 1962, after an infection of the upper respiratory tract he had had pain in the region of the right shoulder and weakness of his arms symptoms which however had more or less disappeared Examination revealed suggillations of the arms and legs as well as muscular atrophy of moderate degree of the arms and some muscular fasciculation Two months later he was referred to the department of internal medicine in Malmo for investigation Then he had no suggillations and comprehensive coagula

tion studies (18) showed nothing abnormal The diagnosis is obscure

Case 8416 (*A H*) A 56-year old man who had for the previous 2 years been disabled by low back pain Apart from this he felt well and physical examination revealed nothing remarkable On two occasions however, the white blood count was 15 200 and 15,700 with 51 % morphologically normal lymphocytes The bone marrow contained 9 % plasma cells and 10 % lymphocytes and roentgen examination showed pronounced spondylosis The diagnosis is obscure, lymphatic leukaemia is suspected but the increased number of plasma cells in the bone marrow remains unexplained

Case 6497 (*K.P*) A previously healthy 75 year-old man was admitted to hospital in September 1964 because of 6 months history of obstipation abdominal tension anorexia loss of weight and fatigue He was slightly jaundiced and ascites was suspected Roentgen examination of the chest stomach colon skull ribs spine and pelvis was unable to explain this condition The serum bilirubin was slightly increased (1 3—2 6 mg/100 ml) and the alkaline phosphatase was markedly increased (45—70 U Buch & Buch normal laboratory range 2—8 U) The patient deteriorated rapidly and died 3 weeks later Post mortem examination was not done Diagnosis neoplasia with involvement of the liver (?)

Case 6350 (*G.S*) An 87 year-old man who had always felt well Palpation revealed no enlargement of the lymph nodes of the spleen or of the liver and general physical examination showed nothing remarkable The W B C was 20 500 The blood smears contained 64 % normal lymphocytes and abundant ghost cells The marrow smears which were poor in cells contained 53 % lymphocytes Diagnosis lymphatic leukaemia

Case 6844 (*E F*) A 73 year old man who had felt well until 1962 when he was subjected to prostatectomy In the spring of 1964 he had been found to have diabetes for which he was treated with chlorpropamide The E S R was then 100 mm At examination in July 1964 he had no symptoms The sternal marrow contained 18 % polymorphous plasma cells and the concentration of the M-component (γA) was 2 4 g/100 ml Roentgen examination of the skeleton revealed no abnormalities Diagnosis myelomatosis

Case 7006 (*F J*) A 67 year old man who after symptoms of biliary disease for some years had been subjected to cholecystectomy in May 1964 He had for some years had chest pain on physical exertion and a few weeks before the present examination he had noticed a rash Status slight dyspnoea B P 200/125 mm Hg pityriasis rosea like efflorescences on the trunk E C G showed signs of coronary insufficiency The sternal marrow contained 19 % polymorphous plasma cells and the concentration of the M component (γA) was 1 0 g/100 ml Roentgen examination of the skeleton revealed no abnormalities Diagnosis myelomatosis

Case 8170 (*I O*) A 76-year old woman who had for 3—4 years been known to have slight diabetes and who had always felt well and now appeared healthy Roentgen examination showed a few rarefactions suggestive of myelomatosis the bone marrow contained 4 % plasma cells and the concentration of the M-components (γA + γG) was 1 2 g/100 ml Diagnosis myelomatosis?

None of the 64 subjects had symptoms or pain suggesting osteolytic lesions None had an increased bleeding tendency of the type seen in macroglobulinaemia Waldenstrom or myelomatosis None of the subjects had any enlargement of the lymph nodes spleen or liver and examination of the eyes had not revealed so-called fundus paraproteinaemicus (3) None had increased

tendency to infection, which is common in pronounced immunoglobulin deficiency

Discussion

Material It is difficult to find out the reasons why some persons fail to attend mass health controls In the present control some of the men in active age were probably prevented by their work, even though the department was open until 8 p m As to the youngest groups, the lack of cooperation was probably due to the fact that they felt well The absences among the aged could probably be explained largely by their poor general condition and a nihilistic attitude Some sick persons surely failed to attend because they were disabled by their diseases and thought control examination unnecessary because they were under medical observation or had been admitted to hospital Persons with myelomatosis probably failed to co operate for such reasons Inquiry at local hospitals and interviews with doctors who knew the population revealed that two persons with myelomatosis were known, a 57-year old man and a 53-year-old woman who had had symptoms for 2 years and 1 year, respectively Neither of them attended the mass health control

Judging from the composition of the material (table I) it may be regarded as largely representative of the population above 25 years in the 4 parishes with reservation for the low percentage of men in the lowest age classes and of persons above 70 years

The frequency of M components was found to be about 1 % M components in low concentration (< 0 4 g/100 ml) and with a paper electrophoretic mobility corresponding to that of α_2, β_1 or β_2 fractions or to the maximum concentration of the gamma globulin fraction of normal or increased concentration may remain undetected by the method used It is therefore probable that the frequency of M components in low concentration was somewhat higher than that found by us The frequency of M components increased with decreasing concentration of the M components but only down to a concentration of 0 3 g/100 ml This may be explained by the above mentioned remarks

M components with the same mobility as the α_2 fraction are rare (12) Apart from such components there is no reason to assume that M components in a concentration of 0 4 g/100 ml or more have been missed The frequency of M components in this range of concentration in our material was 0 6 %

The frequency of M components increased with age The increase appeared to be more pronounced for men than for women, but the difference was not statistically significant except in the highest age class

Unlike the distribution of M components according to immunological type in hospital series, that in the present investigation was not dependent on the conventional indications for paper electrophoresis But as far as the three main types of immunoglobulins are concerned, the distribution did not differ substantially from that in large hospital series (2, 5, 16, 21 28), though the frequency

of γM M components was the lowest observed

The results of the *zinc turbidimetric determination and E S R* show that these methods cannot be regarded as sensitive screening tests for M components in low concentration

Bone marrow findings Paraskevas et al (23) showed that certain properties of the plasma cells in the bone marrow in patients with myelomatosis occurred in a number of cases with M components of γA type Flaming compartment formation or intranuclear inclusions were however, not observed among patients with γG M-components In a later large myelomatosis series Drivsholm and Clausen (6) made similar findings and also in a few cases with γG M component they observed plasma cells with the above mentioned properties In our series the blue grey inclusions with surrounding dark rim were equally common in patients with γG M component as in those with γA M component Apart from these inclusions, the properties under discussion were abnormally pronounced in 39 % of the cases with γA M-component and were not seen among 100 plasma cells in any of the other cases An increased frequency of plasma cells with pale intranuclear inclusions flaming or compartments thus occurred also in subjects with γA M component but probably without myelomatosis, and the properties do not appear to be correlated with the concentration of the M components or the number of plasma cells

Skeletal X ray In the only case with rarefactions of the skull the suspicion of myelomatosis was suported to some extent by the concentration of the M component and by slight increase in the number of plasma cells The finding of osteoporosis among the elderly women was not surprising and does not by itself suggest myelomatosis In the subject with a dubious picture of the glenoid cavity other findings argued against destruction of neoplastic origin

The state of health was largely normal for age (64 years) The commonest diseases were those of aging such as cardiovascular diseases and spondylosis deformans M components have been described in patients with cancer (*e g* 20) and in patients with lymphatic leukaemia (*e g* 1) The material included 2 men operated upon for cancer 6 and 8 years previously one man who died shortly after collection of the samples, probably from malignant neoplasm of the abdomen, and one man with clearcut lymphatic leukaemia in a quiescent stage

None of the 7 with γM M components had clinical symptoms or signs of the type seen in macroglobulinaemia Waldenstrom Some of these symptoms and signs however seem (7) to be dependent on a concentration of the M component, which is much higher than those (0 1—0 6 g/100 ml) found in this series No definite diagnosis could be made in the 73 year-old woman who had for many years had mild anaemia was found to have an M-component (γM) in low concentration, several atypical lymphoid cells and a slightly increased number of mast cells in the bone marrow Early

macroglobulinaemia Waldenstrom was, however, suspected

Myelomatosis was strongly suspected in 3 cases Estimation of the true frequency of myelomatosis in this series requires further observation

Judging from figures for the town of Malmo (33), the incidence of living patients with myelomatosis in a population above 25 years of age is 1—2 per 10,000, which agrees with the frequency (2 cases) in the district studied In this district there must be about 80 cases with M components of the type γG and γA *It appears very unlikely* that all of these persons will develop myelomatosis

Summary

In a population of 6,995 persons above 25 years paper electrophoresis of the serum revealed M components in 64 cases M components were more common in the higher age classes

The concentration of M components was usually low and in only 10 sera did it exceed 1 g/100 ml

The distribution of M components from an immunological point of view was γG 61 % γA 27 % and γM 8 % In 3 sera two M components of different immunological types were seen

The number of plasma cells in the bone marrow was more than 3 % in 23 % of the cases studied

Plasma cells with flaming, compartment formation or intranuclear inclusions (excluding blue grey hyaline surrounded by a dark rim) were more common in patients with M components of type γA than in patients with other types of M components

The X ray appearance was suggestive of myelomatosis in one case

The state of health of the 64 persons was fairly normal for age (64 years) The diagnosis of myelomatosis was strongly suspected in 3 cases None of the patients had symptoms of macroglobulinaemia Waldenstrom Three persons had or had had neoplasms and one had lymphatic leukaemia

Acknowledgement

The Swedish National Board of Health who sponsored the mass health control courteously allowed us to use sera for this investigation

The investigation was supported by grants from Ernhold Lundstroms Stiftelse and Alfred Österlunds Stiftelse Malmo

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Chromosome Aberrations in Human Cells Following Treatment with Imuran

Preliminary Report

By

Mogens Krogh Jensen and Mogens Soborg

During the last few years cytotoxic drugs, in particular azathioprine (Imuran®), have been employed to an increasing degree as immunosuppressives, especially in the treatment of the collagen diseases In this context the possibility that some of these drugs might be carcinogenic must be born in mind The ability of several well known carcinogens, leukaemogens and teratogens to produce chromosome aberrations is well established (1 3) Therefore, it was deemed of interest to investigate the possible chromosome damaging effect of Imuran

Material and methods

Five women with various collagen diseases were studied Bone marrow aspirates were obtained from each patient before and 12 to 24 days after start of Imuran therapy In one patient bone marrow was also studied 145 days after cessation of therapy

The marrow aspirates were treated according to a slight modification of the technique of Tjio and Whang (6) Fifty metaphases from each sample were counted and scored for chromosome aberrations

Results

The results are summarized in table I In patients Nos 1 and 2 the increase in cells with structural abnormalities during therapy is significant (χ^2, 0 05 > p > 0 01) The aberrations found were mostly breaks of the chromatid and chromosome type A few abnormal chromosomes were seen i a a ring-chromosome In patient No 1 chromatid exchanges were seen in three cells

Discussion

The fact that a number of well known carcinogens leukaemogens and teratogens produce chromosome damage suggests that agents with similar effect on the chromosomes may prove to be potentially carcinogenic and teratogenic

Submitted for publication November 11 1965

TABLE I Chromosome aberrations in bone marrow cells during Imuran therapy

Pat no and initials	Date	Total dose of Imuran given (mg)	Per cent cells with aberrations[1]
1 KA	14– 4–65	–	2
	30– 4–65	2 950	20
2 ER	12– 1–65	–	2
	5– 2–65	3 600	12
	3–[2]9–65	5 900	18
3 MC	12– 7–65	–	4
	26– 7–65	1 950	10
4 RM	7– 8–65	–	2
	19– 8–65	1 800	6
5 ES	30–11–64	–	6
	21–12–64	3 300	6

[1] The figures based on the count of 50 metaphases
[2] 145 days after cessation of therapy

The possible carcinogenic risk of Imuran in man cannot be evaluated until after years of observation A possible teratogenic effect in man has not yet been reported but such an effect has recently been observed in mice by Githens et al (2)

Although the clinical efficacy of Imuran therapy is striking in some patients suffering from collagen diseases (4, 5), the findings reported here call for considerable caution in the use of Imuran and related drugs in the management of patients with non neoplastic diseases For the time being it would appear wise to use these drugs only when therapy is imperative and other means of therapy have failed

Acknowledgement

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From the Neurological Clinic (Head Eric Kugelberg M D), Karolinska sjukhuset Stockholm, Sweden

Tegretol, a new Therapy of Tabetic Lightning Pains

Preliminary report

By

KARL EKBOM

Lightning pains are known to represent a most distressing symptom of tabes dorsalis To day no effective therapy exists against these pains, which often persist despite adequate antiluetic therapy

Recently I have used Tegretol® in two tabetic patients, who were completely incapacitated by severe lightning pains Tegretol (Carbamezapine) is chemically related to imipramine (Tofranil) and has an extremely good effect against the pains in trigeminal neuralgia (1) My immediate results against the tabetic lightning pains were very promising and are presented here as a preliminary communication

Case reports

The patients (cases 1 and 2) were both men aged 77 and 59 years respectively Both presented the classical symptoms of tabes dorsalis Argyll Robertson pupils spinal ataxia impaired deep sensibility and loss of deep pain decreased or lost deep tendon reflexes. In case 1 the Wassermann test and the treponema pallidum immobilization test (TPI) were positive in the blood but negative in the spinal fluid This patient had been treated several times earlier with penicillin In case 2 the serological examinations and the TPI test were positive in the blood as well as in the spinal fluid

Since 20—30 years both patients had severe lightning pains several times per day The pains were very short and intensive and were localized to small spots in the lower limbs or the chest or the abdomen In case 1 there were also a pronounced hyperalgesia around the chest

Both were observed at the out patient department 2—3 months prior to treatment with Tegretol They had sever pains every day and were practically never free from symptoms

At the neurological department they were at first observed during a period of 4 days they had several attacks of pain every day and night and required a lot of the usual analgesic drugs In case 1 a constant hyperalgesia produced intensive pains especially during night sleep

Treatment The initial dosage of Tegretol was 200 mg twice daily In *case 1* the pains became less pronounced after 1—2 days of treatment and after 3 days the patient

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was completely free from symptoms. His sleep was undisturbed and he had practically no hyperalgesia. After 11 days the treatment was withdrawn. Two days later the pains and the hyperalgesia were as severe as before the treatment. The patient remained without Tegretol for 3 days after which the treatment was given again. A dosage of 600 mg per day again produced complete freedom from pains within 2 days.

In *case 2* a complete relief from pains was obtained within the first day of treatment. After 5 days Tegretol was withdrawn and the pains returned immediately. During 3 days without tablets he had several attacks of pain every day. Tegretol was then given again in a dose of 600 mg daily which once more resulted in a complete relief from the pains on the first day of treatment.

Discussion

In these two patients the Tegretol medication had an almost immediate effect upon the lightning pains and the hyperalgesia. Both patients became completely free from symptoms within 1—3 days. Upon withdrawal of treatment the pains recurred almost instantaneously. A second period of treatment again resulted in a complete disappearance of pains within 1—2 days.

A similar dramatic effect of Tegretol has been demonstrated in patients with trigeminal neuralgia (1). In fact these pains are similar to the tabetic lancinating pains. Both my patients stated that after having an attack they could easily provoke a new bout of pain through gently touching the skin over the painful area. This phenomenon definitely resembles the trigger zones of trigeminal neuralgia. Consequently there is possibly a similar mechanism for the pains of tabes dorsalis and those of trigeminal neuralgia (2).

Because the tabetic lightning pains are known to be very resistant to all therapy used hitherto, an effective treatment would be of great value. The present results indicate that Tegretol may be highly effective in giving symptomatic relief from these pains. Probably this treatment will also provide some further evidence about the origin of the tabetic pains.

Summary

Two tabetic patients with severe lightning pains were treated with Tegretol in a dosage of 200 mg 2—4 times daily. The therapeutic results indicated that Tegretol has a specific and immediate effect on the lightning pains.

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Latex Slide Test in Thyroid Diseases

By

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The latex slide test has been widely accepted as a screening method for rheumatoid arthritis The test is neither highly sensitive nor specific Cases with other rheumatic diseases, especially those with systemic connective tissue symptoms, frequently react positively On the other hand the test is often negative in the early cases of rheumatoid arthritis

In recent years the unspecific nature of the latex test has become more evident Reports of latex positive non rheumatic cases are numerous Positive latex tests have been found in patients with sarcoidosis (8), with syphilis (4, 10) with viral *infections* (4) *with liver* diseases, especially with hepatitis and cirrhosis of liver (1, 4 7), with malignant tumors (7 11), with diffuse pulmonary fibrosis (12) with sub acute bacterial endocarditis (14) and with myeloproliferative disorders (2)

The purpose of this paper is to add to the list the thyroid diseases in which the latex slide test seems to be positive with an unexpected frequency

Submitted for publication August 30 1965

Material and methods

The original material comprised 100 unselected patients with thyroid diseases However 20 cases with a positive latex slide test and with hyperthyroidism were excluded from the material because they had other concomitant illnesses [pulmonary diseases (5) liver stasis (5) or cirrhosis (1) rheumatoid arthritis (5) or ill defined arthralgias (2) chronic lymphatic leukaemia (1) or sarcoidosis (1)] that could have caused the latex positivity

The majority (53 cases) of the remaining 80 patients suffered from hyperthyroidism (table I) Of these 32 had a nodular goiter and 21 a diffuse one The rest of the material (27 cases) consisted of 14 cases of idiopathic hypothyroidism 3 cases of subacute thyroiditis *one* with *Riedel s thyroiditis and* 9 cases with atoxic nodular goiter The mean age of the patients was 53 years (16—81) 25 patients were over 60 years

The latex slide test (Hyland RA test) was performed on the sera of the patients according to the manufacturer s instructions Results were recorded on a scale of 0 to 3 plus In most of the cases with latex positivity the Waaler Rose test (in 19 cases) and the Hyland TA test for detecting antibody to human thyroglobulin (in 22 cases) were performed The ultracentrifugal serum analysis

TABLE I The latex slide test in cases of thyroid diseases in comparison with healthy controls and with cases of rheumatoid arthritis

Diagnosis	No of cases	Latex slide test					
		+++ or ++		+		—	
		(No)	(%)	(No)	(%)	(No)	(%)
Hyperthyroidism	53	9	(17)	11	(21)	33	(62)
Hypothyroidism	14	4	(29)	3	(21)	7	(50)
Subacute thyroiditis	3	1		1		1	
Riedel s thyroiditis	1	0		0		1	
Atoxic nodular goiter	9	0		0		9	(100)
Healthy controls	65	3	4 6	1	1 5	61	93 9
Rheumatoid arthritis	229	141	61 6	24	10 5	64	27 9

*was performed in three patients with hyper*thyroidism and in one with subacute thyroiditis who had the latex slide test strongly positive

The controls were 65 healthy medical students For comparison our rheumatoid arthritis material is also presented

Results

The results are presented in table I It can be seen that half of the cases with hypothyroidism and about one third of those with hyperthyroidism were latex positive The case with Riedel s thyroiditis and the 9 cases with atoxic nodular goiter reacted negatively In diffuse goiters the latex positivity appeared to be higher than in the cases with nodular goiters We have also performed the latex slide test in 5 cases with hypothyroidism that developed after radioiodine therapy A positive latex reaction was obtained in one of these The percentage of the positive latex slide tests in the control material was 6 1 and in the rheumatoid arthritis group 72 1

The latex positivity was not affected by advancing age in the thyroid material The mean age in the latex positive cases (49 years) was lower than in those which reacted negatively (54 years) In the patients over 60 years of age (25 cases) the latex positivity was 28 %, and in those under 60 years (55 cases) 40 %

Two of the cases in the present thyroid disease material gave a positive Waaler Rose reaction They were both cases with hypothyroidism The Hyland TA test was positive in three cases (one with hyperthyroidism, one with hypothyroidism and one with subacute thyroiditis) The ultracentrifuge experiments on the sera of four cases with a strong latex reaction showed a normal amount of 19 S fraction

Discussion

The latex positivity in our thyroid disease material is clearly *higher than* in the controls The incidence of positive reactions in the latex test is higher in

older age groups (6) In the normal population, 30 % of persons over 60 years old have shown latex positivity (13) The age of the patients had no influence on the latex positivity in the present thyroid disease material

The studies of Lospalluto and Ziff (9) and Franklin (5) have demonstrated the existence of two rheumatoid factors According to Chodirker and Tomasi (3) there are high molecular (19 S) and low molecular (7 S) rheumatoid factors Their preliminary data suggest that the 7 S rheumatoid factor is non reactive towards rabbit gamma globulin in the sensitized sheep cell agglutination test According to our ultracentrifugal data we can suppose that the higher incidence of latex positivity in thyroid diseases is due to 7 S rheumatoid factor This is in agreement with our results that show that the Waaler Rose test was generally negative in latex positive cases The hypothesis may be justified that the positive latex slide tests in thyroid diseases are related to an auto immune phenomenon and are produced in response to the release of an auto antigen possibly localized in the thyroid gland

Summary

The latex slide test was performed in 80 cases with thyroid diseases A further 20 cases who showed latex positivity but suffered from other concomitant diseases were not included in the material The positive reaction was observed in 20 cases out of 53 hyperthyroidism cases, in 7 out of 14 hypothyroidism cases and in 2 cases out of 3 subacute thyroiditis cases The test was negative in 9 cases of atoxic nodular goiter and in one with Riedel's thyroiditis In the latex-positive cases the Waaler Rose test and Hyland TA test were usually negative The ultracentrifugal analysis of four cases with a strong latex reaction showed a normal amount of the 19 S fraction It is suggested that the increased positivity of the latex test in thyroid diseases is related to auto-immune processes in the thyroid gland

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The IXth International Congress of Internal Medicine will be held in Amsterdam, September 7—10, 1966

President of the Congress Professor J G G Borst

Secretary General Dr A H Wiebenga

Correspondence should be directed to The Secretariat of the IXth International Congress of Internal Medicine, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands

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The International Society of Geographical Pathology intends to hold its ninth congress from 7 to 10 September included, 1966, in the Physiological Laboratory of the State University at Leiden, the Netherlands

The main theme of the congress will be *Cardiovascular Diseases*, but the results of an inquiry on myocardial infarction that took place in 11 countries, will also be brought into discussion. Furthermore some sessions will be devoted to different aspects of atherosclerosis

The President of the International Society of Geographical Pathology, Dr H E Schornagel, Superintendent of the Central Pathological Laboratory of the Rotterdam Municipal Hospital acts as *President of the Organizing Committee*, *Vice-president* is Dr A Schaberg, professor of pathological anatomy at the Leiden State Universit[y] *The secretarial address is* 16 Lange Voorhout, The Hague, The Netherlands

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Universite de Paris, Faculte de Medecine *Les Journees Medicales Annuelles* de l'Hopital Broussais la Charite, sous la Presidence du Professeur Pasteur Vallery Radot, Service du Professeur Paul Milliez auront lieu jeudi 5, vendredi 6, samedi 7 mai 1966

Il est recommande de s inscrire assez tot, le nombre des participants etant limité Priere d envoyer des droits d inscription au Centre de Recherches sur l Hypertension Arterielle Professeur Milliez Hopital Broussais 96 Rue Didot, Paris XIV^e (cheque bancaire ou mandat carte) Les droits d inscription sont de 100 F tout compris (ensemble de ces journees et volume des conferences) Un fichet de reduction SNCF sera adresse sur demande

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International Symposium on Major Endocrinic Surgery On the 5th 6th and 7th of May 1966 an International Colloquy will be held in Lyons under the patronage of the National League against Cancer and under the U I C C s auspices at the Palais des Congres about major endocrinic surgery as a treatment for cancer of the breast in an advanced phase

For further information apply to Mr Dutou Centre Leon Berard 28 rue Laënnec Lyon 8^e

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From the Streptococcal Department Statens Seruminstitut and The Medical Department VII
Municipal Hospital Copenhagen Denmark

Leucocyte-specific Anti-nuclear Factors in Patients with Felty's Syndrome, Rheumatoid Arthritis, Systemic Lupus Erythematosus and Other Diseases

By

Viggo Faber and Preben Elling

It is generally accepted that anti nuclear factors (ANF) combine with the nuclei of all mammalian tissues and they thus seem to be non specific with respect to organs as well as species By use of the immuno fluorescent antibody technique with various tissues as nuclear antigen an ANF was demonstrated that seemed to react specifically with the nuclei of the granulocytes but not, for example, with lymphocytes or other mesenchymal cell nuclei nor with epithelial cell nuclei (9) This organ specific ANF was originally found in the serum derived from a patient with rheumatoid arthritis splenomegalia and a severe leucopenia i e the syndrome described by Felty (11)

Since the first demonstration, more than 5,000 sera from patients with various medical disorders have been investigated during routine examinations for ANF Altogether 30 sera with this new leucocyte specific factor were found and this report deals with the clinical and serological findings in these patients

Submitted for publication January 11 1966

Methods

The fluorescent antibody test for demonstrating anti nuclear globulins was performed by means of the indirect technique given by Weller and Coons (20) As nuclear substrate various human tissues were used The blood smears were prepared by the slide method and always used within half an hour The different human tissues employed were obtained in vivo and immediately cut into small pieces they were frozen at — 70 C and stored at this temperature The tissues were cut in a cryostat into 5 μ thick pieces mounted on slides and air-dried for 15 minutes with a fan Each tissue was covered with a few drops of undiluted serum and incubated in a moist chamber for 30 minutes After incubation the serum was carefully washed off in several changes of buffered saline (pH 7 2) for 20 minutes and stained with anti human gamma globulin conjugated with fluorescein isothiocyanate After 30 minutes incubation *the conjugate was washed off and the slides* were mounted in buffered glycerin Fixation was never performed

For routine examinations thyroid glands were used If the presence of leucocyte specific ANF was suspected from this investigation (from the intensity of the staining of the few granulocytes which infiltrate the thyroid tissue) (fig 1) more detailed exami

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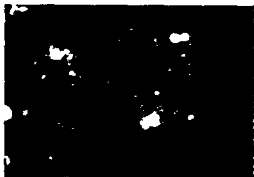


Fig 1 Thyroid section pre treated with a serum containing a leucocyte-specific ANF Nuclear staining only of the infiltrating polymorph nucleated leucocytes is seen.

nations using other tissues as nuclear antigen were performed in order to confirm or invalidate the primary observation The blood smears used were prepared from healthy normal individuals of different blood groups and from patients with lymphatic myelocytic, and stem-cell leukaemia Seven sera were also investigated using blood smears from healthy rabbits rats and mice The other tissues employed were the following normal adult normal foetal and cirrhotic livers parotid glands mucosa of the stomach striated and heart muscles and skin Often 2 or 3 tissues were placed on the same slide

The conjugates were 3 batches of anti human-gamma globulin prepared as described in detail in a previous paper (10) Only antisera showing no precipitating lines other than against human gamma-globulins were conjugated

The microscope was a special Leitz Ortholux (Zernicke) with equipment for combined fluorescence and phase-contrast microscopy with a high pressure mercury vapour lamp (Osram HBO 200 an UG 1-exiter filter and a colourless UV absorbing barrier filter

Absorption experiments Thyroid nuclei were prepared according to the technique described by Hogeboom (14) and used in the form of a 10 % suspension in buffered saline Leucocytes were obtained from a donor of blood group O To blood collected in heparin dextran was added and the mixture was left at 37 C for 45 minutes for sedimentation The buffy coat was removed and frozen at —20° C until required as a 10 % suspension in buffered saline Each serum investigated was "incubated for 24 hours at 4 C with a sediment from a standard volume of one of the suspensions mentioned This procedure was repeated 4 times After incubation the sera were centrifuged and the supernatants were tested for ANF The amount of nuclei used was sufficient to absorb a high titre ANF positive serum from a patient with systemic lupus erythematosus

Sulphydryl sensitivity Before testing for ANF the sera were diluted with equal volumes of neutralized 0 5 M Penicillamine (Dimethyl cystein Distra Products Ltd Liverpool England) and incubated for 2 hours at 20° C Controls were diluted with buffered saline

Enzyme treatment The glass slides of blood smears were incubated for 30 minutes at 20 C with a few drops of a 1 % solution of DNase (Worthington Biochemical Corporation Freehold New Jersey) before testing Control slides were incubated with buffered saline

Leucocyte antibodies were determined according to the method given by Kissmeyer Nielsen and Andresen (15) with use of the anti globulin consumption test They were performed by Kissmeyer Nielsen M D (Blood Grouping Laboratory Kommunehospitalet Aarhus) Leucocyte agglutinins were determined according to the method given by Dausset and Nenna (7) They were performed by Mrs Messeter M D (Blood Grouping Department Statens Seruminstitut Copenhagen)

Rheumatoid factors were determined by the sheep-cell agglutination technique (Waaler Rose reaction) in a slight modification described by Bichel et al (3) and with use of latex particles sensitized with gamma-globulin (Hyland reagent)

Materials and results

Fifteen of the 30 sera containing a leucocyte specific ANF were found

among 5 000 sera received in the laboratory for routine examinations of universal reacting ANF In addition 558 other sera from patients with well known diseases were investigated, and among these a positive reaction was given by 4 of 100 sera from patients with rheumatoid arthritis 8 of 8 sera from patients with Felty s syndrome 2 of 50 sera from patients with S L E, 1 of 40 sera from patients with myelomatosis while the leucocyte specific ANF was not found in any sera from 100 patients with hepatic cirrhosis, 100 patients with pernicious anaemia 50 patients with ulcerative colitis, 20 patients with sarcoidosis 30 patients with leucoses or in any sera from 25 patients with leucopenia of various causes The diagnostic criteria used for the classification of most of these diseases were given in a previous paper (10)

Some clinical details of these 30 patients are given in table I All sera derived from patients with Felty s syndrome so far investigated contained the leucocyte specific ANF In 2 of these 8 patients splenectomy had been performed 8 and 10 years respectively, before the time of collecting the serum specimens One of the patients did not exhibit granulocytopenia but had been classified as a definite case of Felty s syndrome before the operation All patients but one were treated with steroids

The leucocyte specific ANF was furthermore demonstrated in altogether 14 patients (10 females and 4 males) who had suffered from classical rheumatoid arthritis for 3 to 30 years Four of these 14 patients showed granulocytopenia, in none of the patients was splenomegalia demonstrated Some patients also suffered from other diseases as entered in the table Eight patients received steroids

Three sera from patients with systemic lupus erythematosus or a lupus-like syndrome contained the leucocyte-specific ANF In case 23 clinical, biochemical, and serological signs of classical S L E were found, and the patient had been treated successfully with steroids for 4 years until 1962 in the last two years signs of activity had been present only in the skin, but intermittently positive L E cell phenomenon was found, when the serum specimen was drawn no leucopenia or splenomegalia could be demonstrated Case 24 developed a lupus like syndrome with arthralgia recurrent febrile episodes pleural and pericardial effusions, cutaneous eruptions and leucopenia during treatment with Propylthiouracil given on account of a hypermetabolism Case 25 had been suffering from arthralgia and recurrent febrile episodes for a few months she was leucopenic on several occasions but the spleen was not palpable on account of several strongly positive L E cell preparations and negative tests for rheumatoid factors she was classified as a case of probable systemic lupus erythematosus

Case 26 had been well except for a diabetes mellitus treated with Tolbutamide for three years She was admitted to hospital on account of diffuse pains in her back and radiological and cytological evidence of myelomatosis was found A M component was demonstrated in the serum On admission she

TABLE I Some clinical details of 30 patients in whose sera leucocyte specific ANF was found

No	Age	Sex	Diagnosis	Duration (years)	Leucocytes (mm³)*	Steroid	Remarks
1	59	♀	Felty's syndrome	3?	1 600	+	
2	72	♀	Felty's syndrome	12	4 100	+	
3	73	♀	Felty's syndrome	30	1 700	+	Thrombocytopenia
4	61	♀	Felty's syndrome	33	900	+	Diab mell
5	43	♂	Felty's syndrome	10	9 000	+	Splenectomy 1954
6	78	o	Felty's syndrome	20	4 500	+	Splenectomy 1956
7	54	o	Felty's syndrome	22	1 500	+	
8	70	♂	Felty's syndrome	32	3 000	+	
9	52	♀	Rheum arthr	>10	3 000	0	
10	70	♀	Rheum arthr	3	3 000	+	Dermatomyositis mb Basedow
11	40	♀	Rheum arthr	9	3 ?00	0	
12	58	♀	Rheum arthr	16	3 700	+	Polyneuritis
13	77	♀	Rheum arthr	20	5 200	+	Recid med allergy
14	59	o	Rheum arthr	30	5 500	0	
15	54	♀	Rheum arthr	11	6 300	+	Rec d rt cana
16	65	♀	Rheum arthr	>10	6 600	+	
17	49	♀	Rheum arthr	>10	5 700	0	
18	58	♀	Rheum arthr	3	6 700	+	
19	50	♂	Rheum arthr	4	8 400	+	
20	46	♀	Rheum arthr	20	9 3?0	+	
21	52	o	Rheum arthr	20	9 400	0	Diab mell
22	54	o	Rheum arthr	4	10 040	+	
23	29	♀	S.L.E.	7	6 600	0	
24	14	♀	S.L.E.	1	3 660	0	Hyperthyroidism Lupus e propylthiouracil
25	25	♀	S.L.E. o p	1/2	3 000	0	
26	70	♀	Myelomatosis	1 2	3 000	0	Diab mell
27	77	♂	Diab mell	19	4 840	0	Nephropathia retinopathia polyneuropathia
28	58	♂	Hyper glob	10	7 300	0	
29	65	♀	Collagenosis	?	1 920	+	Recurrent infections
30	60	♀	Collagenosis	?	1 080	0	

Except for patients nos 1 2 3 4 7 8 and 24 no splenomegalia was found

* In all patients with leucopenia this was due to a granulocytopenia

was leucopenic with a hypercellular bone marrow A moderate renal impairment was present She was treated with a nitrogen mustard derivative (Alkeran) but developed a pancytopenia and died from bacteriaemia

Case 27 had been treated with insulin for 19 years The diabetes mellitus was complicated with diabetic retinopathia polyneuritis and nephropathia with albuminuria No joint lesion no palpable splenomegalia and no haematological

TABLE II Serological details of 30 patients in whose sera leucocyte specific ANF was found

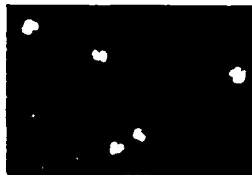
Case no	Diagnosis	L E cells	ANF[1]	Sheep cell agglutinins	Leucocyte agglutinins	Coombs test[2]	Granulocytopenia
1	Felty s syndrome	0	0	0	0	+	+
2	Felty s syndrome	—	+	—	—	—	+
3	Felty s syndrome	+	+	+	0	+	+
4	Felty s syndrome	—	+	—	—	—	+
5	Felty s syndrome	—	+	+	—	—	0
6	Felty s syndrome	—	+	0	—	—	+
7	Felty s syndrome	(+)	+	+	0	(+)	+
8	Felty s syndrome	—	+	+	0	0	+
9	Rheum arthr	—	+	+	—	—	+
10	Rheum arthr	+	+	+	0	0	+
11	Rheum arthr	0	+	0	0	(+)	+
12	Rheum arthr	—	+	+	0	+	+
13	Rheum. arthr	0	+	+	—	—	0
14	Rheum arthr	0	+	+	0	0	0
15	Rheum arthr	—	0	0	+	+	0
16	Rheum arthr	0	+	+	—	—	0
17	Rheum arthr	—	+	+	—	—	0
18	Rheum arthr	0	0	+	+	—	0
19	Rheum arthr	—	0	+	0	0	0
20	Rheum arthr	0	0	+	—	0	0
21	Rheum arthr	0	+	+	0	0	0
22	Rheum arthr	—	+	+	—	—	0
23	S L E	+	+	0	—	—	0
24	S L E	0	+	0	0	0	0
25	S L E o p	+	+	0	0	—	+
26	Myelomatosis	0	0	0	0	(+)	+
27	Diab mell	0	0	0	0	0	0
28	Hyper γ-glob	0	+	+	0	—	0
29	Collagenosis	0	+	+	0	—	+
30	Collagenosis	0	+	+	0	0	+

[1] Universal reacting ANF

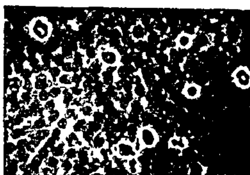
[2] Coombs consumption test with leucocytes

disorder except for a slight anaemia was found

The last 3 patients in whose sera the leucocyte specific ANF was found showed rather indistinct symptoms, and no definite diagnoses were established Case 28 was admitted to hospital owing to recurrent infections in the respiratory tract Signs of pancreatic (and hepatic?) disorder and hyperglobulinaemia were present Case 29 and 30 had been treated with Prednisone on account of



A



B

Fig 2 A Blood smear pre treated with a serum containing a leucocyte specific ANF B Phase contrast of the same field Nuclear staining only of the 5 granulocytes but not of the 2 lymphocytes is seen

arthritic pains and intermittent granulocytopenia Both patients had been suffering from recurrent infections

The serological details of the patients are given in table II Investigations for L E -cell phenomenon were performed in 19 of these patients and found positive in 2 patients with Felty s syndrome, 1 patient with rheumatoid arthritis and 2 patients with S L E

In the sera of 7 patients in this series the leucocyte specific ANF was the only anti nuclear factor found (1 patient with Felty s syndrome, 4 with rheumatoid arthritis, the patient with myelomatosis and the one with diabetes mellitus (case 27) The sera from the other 23 patients contained organ and species non specific ANF as well

The sheep-cell agglutination test was performed on 28 sera and positive results were obtained with 4 Felty sera, with 12 sera from patients with rheumatoid arthritis, and with sera nos 28 29 and 30 No agglutination was given by the sera from 2 Felty patients 2 patients with rheumatoid arthritis 3 patients with S L E and cases 26 and 27 All sera were also investigated for rheumatoid factors as determined by the streptococcal agglutination test, but this gave only one more positive serum (case 15) With the latex test all sera from the patients with Felty s syndrome and rheumatoid arthritis were positive except for case 2

Leucocyte agglutinins were seldom found Out of the 19 tests performed only 2 were positive (nos 15 and 18) neither of these patients were leucopenic In the sera from 7 patients with leucopenia no agglutinins could be demonstrated

With use of *Coombs indirect consumption test* leucocyte antibodies were found in 4 of altogether 16 sera investigated (2 from patients with Felty s syndrome and 2 with rheumatoid arthritis) dubiously positive results were found in 3 patients (1 Felty s syndrome 1 rheumatoid arthritis and 1 myelomatosis) Six of these 7 positive sera were derived from patients with granulocytopenia

Investigations for species and organ specificity When different human tissues were

used and sera nos 1, 15 18 19 20 26 and 27 were investigated only the nuclei of the granulocytes infiltrating the tissues were stained With use of blood smears only polymorph nucleated cell nuclei were stained (fig 2) These 7 sera also stained the polymorph-nucleated leucocytes from rats while none of the white blood cells from rabbits or mice were stained When the other 23 sera were investigated all human cell nuclei were stained, but those of the granulocytes showed a brighter fluorescence

Details of the following investigations will be given in later reports (8) but the preliminary results are summarized in the following

Absorption studies Seven sera were investigated With the amount of thyroid nuclei used the universal reacting ANF was removed in all sera leaving the leucocyte specific ANF unaffected except in 3 sera in which a slight fall in titre was observed Using leucocytes as absorbent, the leucocyte specific ANF were removed completely in 6 sera and partially in one serum, but this treatment also reduced the activity of the universal reacting ANF

Sulphydryl sensitivity Ten sera were investigated (nos 1 3, 6, 7 8 9 10 15 19 and 26) All leucocyte specific ANF were apparently resistant to treatment with Penicillamine as also was the case with most of the "universal reacting ANF In sera nos 6 and 10 Penicillamine was not able to split the leucocyte specific ANF while this treatment partly removed the activity of the "universal reacting ANF leaving behind a speckled ANF

Treatment with DNase abolished the reactivity of the polymorph nucleated nuclei with all 7 sera containing only the leucocyte specific ANF However, treatment of the blood smears with buffered saline also inhibited the positive reaction in 2 cases

Discussion

Although it is evident that certain nuclei are preferable for studying the different staining patterns of the universal reacting ANF (2 5 18) the choice of tissue is generally considered unimportant the anti nuclear factors apparently lacking organ as well as species specificity This assumption may partly be due to the fact that most sera used as controls in these investigations have been high titred sera from patients with systemic lupus erythematosus for which this non specificity certainly holds good In only a few studies have nuclei been shown to differ in their antigenic capacity (12 13, 17 19) Hijmans et al (13) furthermore stress the significance of a proper selection of antisera used for conjugation (see also 1)

As mentioned in a preliminary report (9) the factor here described has probably been demonstrated before (4) though the organ specificity of the factor was not demonstrated The serological investigations performed in the present report indicate that the ANF reacting with granulocyte nuclei only is not identical with the ordinary universal reacting ANF In the absorption studies only the leucocytes were able to remove the leucocyte specific ANF while this factor remained in the sera after absorption

with thyroid cell nuclei The distinction betweeen the two factors was also supported from treatment of some of the sera with Penicillamine Investigation of the factors by the use of various human and animal tissues stresses the differences still more

The finding of a factor specifically directed against the nuclei of the granulocytes raises the question of what specific antigens are present in these nuclei In peripheral blood smears the antigen is rather unstable, the reactivity being weakened within half an hour, whereas the antigen in the leucocytes of the tissues that have been frozen and stored at — 70° C seems stable Probably on account of quantitative differences in the nuclear antigen, the fluorescence of the leucocytes in the tissues is more intense than that of those in the simultaneously used peripheral blood smears In the latter, the best reacting antigen is obtained when they are prepared with a minimum of damage to the leucocytes The fluorescence of the leucocytes was in all but two cases homogeneous, this as regards the ordinary ANF, should indicate an antibody reacting with the desoxyribonucleoprotein complex of the nuclei In two cases fluorescence of the periphery of the nuclei was observed which should indicate an antibody directed against DNA Since the reactivity of the leucocytes was abolished by treatment with a DNase preparation, DNA is probably involved in the reaction, but no conclusive evidence can be drawn from these few experiments

Thyroid glands or other human tissues infiltrated with leucocytes are well fitted for the screening of sera to determine a leucocyte specific ANF Generally the presence of this factor is easily recognized when high titred sera are investigated, but the simultaneous use of phase contrast microscopy has been of invaluable help in distinguishing the cell type stained, especially when blood smears are used as antigen source A leucocyte specific ANF may be missed if the staining is weak or if the tissue contains only few leucocytes If a serum contains a "universal reacting' ANF in equal or higher titer as well, special procedures, e g absorption, blocking with specific antisera, use of different conjugates, etc, are necessary in order to disclose the factor When, with use of blood smears alone as antigen, sera containing both antinuclear factors are investigated, there is only a small difference, if any, between the intensity of the staining of the nuclei of the granulocytes and that of the mono-nucleated cells, and it is advisable to use tissue sections and blood smears on the same slide

As most of the more than 5 000 sera investigated in the present report were procured for the purpose of determining a universal reacting' ANF, they no doubt form a selected material, comprising mostly patients with various mesenchymal or 'auto immune diseases Included in the material however, are 500 sera from patients with gastric diseases (ulcers and malignant tumours) with no overt mesenchymal diseases

In view of the above mentioned technical circumstances and the composition of the material, the fact that 27 of the 30 sera containing the leucocyte specific ANF were from patients with

rheumatoid arthritis or with some other kind of arthritic lesions suggests a possible predisposition of these patients This applies especially to the patients with Felty s syndrome, all of whose sera contained this factor, and though only 8 patients have been examined it may well be a pathognomonic factor of this syndrome Since leucopenia in patients who later develop a complete Felty's syndrome may be present several years before splenomegalia is demonstrable clinically, it is possible that also some of the 14 patients with rheumatoid arthritis in the present material especially those with leucopenia may eventually be classified as Felty patients The factor may thus be of prognostic significance

The finding of the factor in 3 sera from patients with systemic lupus erythematosus or lupus like syndrome calls for further investigations of S L E sera after absorption, blocking etc to see whether there are any other sera with this factor its presence may have been concealed by the universal reacting" ANF From a serological point of view the findings here reported may support the idea that Felty patients constitute a connecting link between S L E and rheumatoid arthritis, which also appears from the demonstration of the universal reacting ANF in 7 of the 8 Felty sera an incidence otherwise found only in patients with S L E

The leucocyte specific ANF has not been found in any of the patients with hepatic cirrhosis pernicious anaemia or leukaemia complicated with leucopenia and splenomegalia but since only thyroid sections have been used in screening most of the sera in the present material, it is evident that no exact information about the prevalence of the factor in question has been obtained More sera from patients with leucopenia and/or splenomegalia of various aetiologies should be investigated, with supplementary use of blood smears as antigen

Further studies of the possible relationship between this factor and other leucocyte specific antibodies are also necessary, especially with reference to the leucocyte antibodies determined by Coombs indirect consumption technique which was found positive in 8 of the 16 sera investigated The so-called "pseudo LE celll phenomenon found in many cases of drug allergic reactions (16) might for example be related to or be identical with the factor here described

The cause of the leucocyte specific factor is unknown Iso immunization during pregnancy or transfusions of blood is in most cases improbable or impossible The idea of a drug induced formation of antibodies through a haptenic mechanism may possible be suggested from case 24 who developed a lupus like syndrome with leucopenia after treatment with Propylthiouracil but the lack of a serum specimen before the drug medicamentation was started and the incomplete information about other drugs given make such considerations purely hypothetical The auto antibody nature of the leucocyte specific ANF is likely since the factor reacts with the granulocytes from the patients themselves and unlike most drug induced leucocyte agglutinins is a com

plete antibody, not depending on the presence of complement

In patients with Felty's syndrome the leucopenia has been explained as being due to some toxic products formed during recurrent infections or to the presence of drug induced leucocyte agglutinins, but these leucocyte antibodies are not always found, and the withdrawal of the offending drug is seldom followed by an increase of the leucocyte count Since neutropenia may disappear after splenectomy in Felty patients the spleen has been considered to be the cause — through excessive sequestration and phagocytosis of the leucocytes (21) or through an inhibitory substance produced by the spleen marrow (6) To these proposed explanations the leucocyte-specific ANF may be added as a possible contributory pathogenic factor, as was also suggested by Calabresi et al (4) This hypothesis may be supported by the fact that in one of the splenectomized patients in the present report the *leucopenia persisted after operation* However, the other splenectomized Felty patient showed a normal count of neutrophils and still had a leucocyte specific ANF in her serum and in the sera of 14 of the remaining 28 patients in this series no leucopenia was present Furthermore, in some of the patients who have been followed serologically for more than a year, no strict correlation has been found between changes in the titres and decrease or increase of the leucocyte count and unknown qualitative differences of the leucocyte specific ANF have to be postulated if a direct cytopathogenic role is to be suggested for the factor concerned

Summary

With use of Coons indirect immunofluorescence technique, 30 sera containing an anti nuclear factor reacting specifically with polymorph nucleated leucocytes have been found Twenty seven of these 30 sera were derived from patients with arthritic lesions, 8 of whom had Felty's syndrome, 14 had rheumatoid arthritis, 3 had systemic lupus erythematosus, and 2 had febrile episodes and arthritis One patient with myelomatosis and leucopenia, 1 patient with diabetes mellitus and 1 with *uncharacterized symptoms with hyper-*globulinaemia also contained the factor in their sera Sixteen of the patients exhibited leucopenia Some clinical and serological details are given The aetiology and possible pathogenic significance are discussed

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Urinary Excretion of Histamine in Patients with Various Muscular Disorders

By

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If histamine is administered parenterally in man a higher proportion is excreted in the urine in the free form than in the conjugated form (1) The inverse proportion however, is found after peroral administration of histamine (1 2, 18) It has, therefore been considered that in man a low free/conjugated histamine ratio implies intestinal derivation of the histamine (11)

Conversely, there is some evidence in support of the concept that conjugated histamine is formed in the tissues outside the gastrointestinal tract e g after subcutaneous or intravenous injection of ^{14}C histamine in man a small fraction is acetylated (14 15) Furthermore, in patients with urticaria pigmentosa, where histamine is released from tissue mast cells there seems to be an increased urinary excretion of both free and conjugated histamine (5 7)

In a preliminary communication it was reported that patients with dystrophia myotonica show an increased urinary output of histamine particularly of the conjugated variety (17)

Skeletal muscle like all other tissues examined, contains histamine In mice at least dystrophic muscles seem to contain more histamine than normal skeletal muscles (4) In any event, the quantities found in muscular tissue are minute (8) If therefore the pattern of metabolism after subcutaneous injection of ^{14}C histamine (15) reflects the pattern following release, degeneration of muscular tissue would hardly result in any appreciable elevation of urinary histamine levels

The balance of evidence seems to favour an intestinal origin of the excess urinary histamine in patients with dystrophia myotonica However it can not be entirely excluded that the histamine is of endogenous origin and that the histaminuria might be related to the muscular disorder per se This possibility has also recently been mentioned by Bois (4)

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To test this further the urinary excretion of free and conjugated histamine has been estimated in various forms of muscular disorders

Material and methods

Seventeen patients with skeletal muscle disease 8 females and 9 males who had all been admitted to the Department of Neurology University Hospital Rikshospitalet were studied The patients displayed varying degrees of muscular wasting Some maintained a full time job others were completely bedridden The diagnosis was established by clinical evaluation electromyography (EMG) and muscle biopsy, EMG being performed in all cases and biopsy in 11 cases Sex age and diagnoses are presented in table I Three patients in all were receiving anabolic steroids at the time of the study (see table I)

The patient with myoglobinuric myopathy was available for study only during a remission Myoglobinuria is however not extraordinarily rare among horses in the woodland districts at Easter time (the farmers call it Easter disease) At Easter we thus had the opportunity to examine a urine specimen from a horse which according to the local veterinarian was suffering from a moderate but definite attack of myoglobinuria It is noteworthy that this urine specimen could not be examined until 5 hours after catheterization and that the pH at arrival was 6 8 despite the addition of the usual amount of hydrochloric acid to a urine volume of only 210 ml Unfortunately recovery studies were not performed with this particular sample Recoveries of histamine and N acetyl histamine (in quantities equivalent of 10 9 μg base of both) added to urine from a normal horse were 39 % and 51 % respectively

Two patients with acute myocardial infarction of 2—3 days duration were also included in this study These patients both received Dilaudid® the day prior to the day of urine collection The serum glutamic oxalacetic transaminase levels rose to 109 and 416 units respectively, in these patients

In 8 patients with skeletal muscle disease the urinary contents of creatine and creatinine were measured Urinary excretion of creatinine was consistently low normal or diminished in accordance with the usual pattern in muscular dystrophy (9) Creatinuria was present (see table I) in all patients with muscular dystrophy examined except patients nos 11 and 12

Twenty four hour urine samples were collected from all patients The urine was either passed directly into chemically clean 1 litre plastic bottles or transferred as soon as possible after voiding to such bottles which contained 60 ml of 1 2 N hydrochloric acid in order to maintain a pH of < 3.5 The patients were carefully instructed regarding the necessity of complete urine collection During collection the bottles were stored at 4° C and the extraction procedure was started within a few hours of completing the collection The extraction procedure was performed as described in detail elsewhere (16), using the method of Dunér and Pernow (6) Histamine was adsorbed on an *Amberlite IRC-50 column and eluted with* hydrochloric acid

Conjugated histamine represents the increment in free histamine after acid hydrolysis (2) For the adsorption of histamine in the tests for total (total = free + conjugated) histamine columns of 1 0 by 20 0 cm of Amberlite IRC-50 were used in all cases The eluates were stored at 4 C for no longer than 3 days before assay This was performed conventionally on an isolated segment of guinea pig ileum suspended in a bath with Tyrode s solution at 37 C, with atropine sulphate added to a final concentration of 10^{-7} M

All figures for both free and conjugated histamine are expressed in terms of the free base and represent the mean of duplicates Mean recoveries of histamine diphosphate and N acetyl histamine added to urine were 72 % and 74 % respectively No corrections were made for histamine lost during the extraction procedure

TABLE I Urinary histamine excretion in patients with skeletal muscle disease

Case no	Age	Sex	Diagnosis	Creatinine (mg/24 hours)	Creatine (mg/24 hours)	Histamine values in µg base/24 hours: Free	Histamine values in µg base/24 hours: Conjugated	Drugs[1]
1 a	39	♂	[2]M d	–		7	950	Durabol®
1 b						7	2 300	
1 c						9	1 000	
1 d						7	1 300	
2 a	44	♀	[2]M d	617	131	40	51	–
2 b						9	40	
3	41	♀	[2]M d	–		2	32	–
4	55	♂	[2]M d	675	165	9	13	–
5	15	♂	[2]M d	280	440	9	22	–
6	14	♂	[2]M d	–		10	6	–
7	32	♀	[2]M d	–		4	28	–
8	25	♀	[2]M d	640	362	8	3	–
9	46	♂	[2]M d	1 113	442	5	29	–
10	19	♂	[2]M d ? Progr muscular atrophy[3]	740	408	<3	55	–
11	39	♀	[3]K W W	450	18	1	16	Durabol®
12 a	20	♀	[3]K W W	1 140	84	47	15	–
12 b						10	53	
13	19	♂	[4]C-M T	–		9	12	–
14	43	♂	[4]C M T	–		9	41	–
15	54	♂	[4]C M T	–		17	21	Dianabol®
16	59	♀	[4]C-M T	–		15	49	–
17	48	♀	Myoglobinuria	–		11	53	–
					Means	10 2±1 9	28 8±4 3	

The results from patient no 1 are not included in the statistical calculations (see text)

[1] Drugs received during collection of urine

[2] Muscular dystrophy

[3] Myatrophia nevrogenes proximalis (Kugelberg Welander Wohlfart)

[4] Charcot Marie Tooth

Antihistamine (Allergin®) was not used routinely However it was used during the assay of the urinary extracts from some patients and in those cases it completely inhibited the contracting ability of the extracts.

Faecal excretion of free and conjugated histamine like activity was examined in patient no 1 using a method described in detail elsewhere (16)

No dietary restrictions were imposed on the patients prior to or during the study

Results

The results of the studies in skeletal muscle disease in man are summarized in table I In patient no 10, where the free excretion is given as < 3 0 μg base/24 hours, the said value was used in the statistical calculations The mean excretion of free histamine in the control material (16) and in the patient group was 12 6 ± 0 9 (S E of mean) μg/24 hours and 10 2 ± 1 9 μg/24 hours, respectively These values are not significantly different (P > 0 2, Student's t test)

The mean excretion of free histamine in the subgroup of muscular dystrophy was 8 9 ± 2 3 μg/24 hours (Statistical significance versus control excretion P > 0 1) The mean excretion in the subgroup of Charcot Marie Tooth's disease was 12 5 μg/24 hours

Two patients (nos 2 and 12) showed free histamine values exceeding the control range On repeat study however normal levels of urinary histamine were found in both cases

The average excretion of conjugated histamine in the control group (16) was 30 0 ± 5 9 μg/24 hours, against an average excretion of 109 4 μg/24 hours in the study group The excretion of conjugated histamine in patient no 1 (see table I) was constantly elevated and of the same magnitude as encountered in some patients with dystrophia myotonica (17) This patient seemed to be an exception in this material The excretion of conjugated histamine in this case was probably not secondary to the muscular disease itself since all the other patients showed excretion of conjugated histamine within the control range It may, therefore, be proper to exclude this patient from the statistical calculations The average 24 hour excretion would then be 28 8 ± 4 3 μg

The means of conjugated histamine in the subgroups of muscular dystrophy and Charcot Marie-Tooth s disease were 22 4 ± 5 5 μg/24 hours and 30 8 ± 9 9 μg/24 hours, respectively No significant difference existed between either of these subgroups compared with the control excretion, or between the subgroups themselves (P > 0 5) Reservation is taken for the limited number of patients in the subgroups

There was no evident correlation between the degree of muscular involvement and the excretion of both free and conjugated histamine

The excretion of both free and conjugated histamine was normal in the patient with myoglobinuria In 50 ml aliquots of urine from the horse with an acute attack of myoglobinuria no trace of free or conjugated histamine could be detected Neither could free or conjugated histamine be detected in the urine from a normal horse

The excretion of free and conjugated histamine in the 2 patients suffering from acute myocardial infarction were free histamine, 2 1 and 1 0 μg/24 hours, conjugated histamine 26 2 and 12 8 μg/24 hours These patients thus seemed to have a low excretion of free histamine

The faecal contents of free histamine like activity in patient no 1 on two occasions weeks apart, were 24 8 and 30 0 μg/g wet weight (corresponding to urinary tests 1 c and 1 d table I) These values are far above the control range only minute quantities being

present in normal faeces (16) Antihistamine (Allergin®) completely counteracted the ability of the faecal extracts to cause contraction No conjugated histamine like activity could be demonstrated by the method used (see 16)

Discussion

Although the creatinuria indicated that muscular degeneration was taking place at the time of study normal levels of urinary histamine were found in patients with muscular dystrophy and myatrophia nevrogenes proximalis This is taken as *evidence that muscular degeneration as* such does not cause increased urinary histamine levels However, the rate of muscular degeneration could be too slow to give any discernible rise in histamine output in these patients at the time of study

It was therefore felt worthwhile to study patients with acute attacks of myoglobinuria The single patient with this ailment showed normal excretion, but was in remission, and exercise failed to produce any muscular symptoms Because of the paucity of human cases, a horse with an acute attack of myoglobinuria was studied No histamine was found in the urine This can probably not be attributed to the poor recovery of histamine The possibility that histamine was poorly recoverable from the specific sample with myoglobinuria however cannot be completely rejected since the presence of myoglobin (or other substances) might have influenced the assay of histamine

Furthermore, two patients suffering from acute muscular damage of an essentially different nature, viz acute myocardial infarction, did not have increased histamine values in the urine *It may thus be stated that muscular* disease per se does not give rise to high levels of conjugated histamine in the urine The acetylhistaminuria observed in dystrophia myotonica (17) is, therefore, probably not secondary to the degeneration of voluntary muscles, which is an integral part of this disease

Patient no 1 (table I) seems to be an exception in this series The high excretion of conjugated histamine in this patient was probably not caused by *the administered anabolic steroids as* patients nos 11 and 15 also received such therapy and had normal excretion (see table I) Furthermore samples I c and I d (see table I) were obtained 6 and 12 weeks, respectively, after the steroid treatment was stopped

It is, however, noteworthy that high histamine like activity was detected in the faeces from this patient Bronchial asthma (3, 10 12) and cardiac disease (13) are the only two disorders in which faecal histamine levels of this magnitude have been found The finding of increased faecal excretion of histamine in cardiac disease is of special interest in this connection since 2 patients with acute myocardial infarction were included in this series Unfortunately, faecal excretion of free and conjugated histamine like activity was not examined in these patients

The findings in patient no 1 (table I) suggest that there is an association between histamine like activity in the lower part of the alimentary tract and conjugated histamine in the urine It

seems unlikely that this association could be incidental. There is reason to believe that the high urinary excretion of histamine is not occasioned by the muscular disorder in this patient either. Future research, however, will be directed towards clarifying this point.

Summary

Sixteen of 17 patients with various forms of skeletal muscle disease associated with degeneration showed urinary excretion of free and conjugated histamine within the control range. The previously observed increased excretion of N-acetylhistamine in dystrophia myotonica is therefore probably not secondary to muscular degeneration as such.

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Hydroxyproline in the Serum and Urine

Normal Values and Clinical Significance

By

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Urinary hydroxyproline (HP), which is considered to be an index of collagen metabolism, has recently been the subject of intensive investigation Alterations in the urinary total HP values have been demonstrated in several diseases and experimental conditions In addition, it has been suggested that HP assays would be of value in the diagnosis of some diseases and in following their response to treatment (4, 9, 11 15, 23, 33) The purpose of this study has been to determine the normal values for urinary total HP and serum free HP to compare these values with those found in several diseases and to discuss the clinical importance of determinations of HP in the urine and serum

Clinical material

Control data (tables I and II) were obtained from 46 healthy volunteer medical students and 49 hospitalized patients without any active disease that could be considered to affect collagen metabolism There was no significant difference between the HP values of the medical students and those of the hospitalized patients of the same age group The patients with various diseases (table III) were typical cases and the diagnoses were based on the clinical picture and laboratory findings The 21 year-old woman with Turner s syndrome and the 20-year old man with Klinefelter s syndrome both had high urinary pituitary gonadotropin values. The patients with malabsorption syndrome were all women and they were 17, 18 and 68 years of age In the two young malabsorption patients there was roentgenographic evidence of delayed maturity In all three cases there were disturbances in calcium metabolism and in the tocopherol loading tests Further morphological changes were present in the intestine (villus atrophy diverticulosis) Azotaemia was in one of the patients due to chronic glomerulonephritis and in the other due to renal amyloidosis Both these patients had greatly elevated serum creatinine values

Methods

The patients and control subjects were on a low gelatin diet (no gelatin meat or fish) for 24 hours before collection of the urine and further for the 24-hour period during which the urine was collected The urine was

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TABLE I Urinary hydroxyproline in normal subjects

Age (yrs)	Group	No	Total hydroxy proline (mg/24 hrs/m²)		Total hydroxy proline (mg/24 hrs)		Free hydroxy proline (mg/24 hrs)	
			Mean	Range	Mean	Range	Mean	Range
18–21	♂	14	21.1	15.4–28.3	39.7	29.6–55.1	0.74	0.49–0.95
	♀	8	17.0	13.4–21.2	26.8	20.3–36.5	0.71	0.36–1.12
	All	22	19.6	13.4–28.3	35.0	20.3–55.1	0.73	0.36–1.12
22–40	♂	18	15.3	9.2–21.0	28.7	18.0–42.4	0.55	0.34–0.75
	♀	10	14.7	9.6–23.8	21.7	15.1–40.8	0.71	0.41–1.33
	All	28	15.1	9.2–23.8	27.3	15.1–42.4	0.61	0.34–1.33
41–55	♂	15	14.0	9.7–23.4	15.4	15.2–42.8	0.65	0.30–1.23
	♀	5	12.9	10.7–16.0	21.1	15.9–28.5	0.46	0.33–0.64
	All	20	13.7	9.7–23.4	24.3	15.2–42.8	0.60	0.30–1.25

TABLE II Serum free hydroxyproline as μg/ml in normal subjects

Subjects	N	18–21 years		N	22–40 years		N	41–76 years	
		Mean	Range		Mean	Range		Mean	Range
♂	26	1.07	0.73–1.50	22	1.13	0.88–1.51	15	1.03	0.63–1.52
♀	12	1.01	0.77–1.32	12	0.96	0.75–1.20	8	0.97	0.70–1.44
All	38	1.05	0.73–1.50	34	1.07	0.75–1.51	23	1.01	0.63–1.52

TABLE III Normal values for urinary free and total hydroxyproline and serum free hydroxyproline. The values are calculated with the aid of the formula $\bar{x} \pm 2S$ after logarithmic transformation of the hydroxyproline values. Urinary total hydroxyproline is expressed as mg/24 hours/m² urinary free hydroxyproline as mg/24 hours and serum free hydroxyproline as μg/ml

Group	No	Total urinary HP	Free urinary HP	Serum free HP
Normal adults				
18–21 years of age	22	13.0–28.0		
22–55 years of age	48	8.5–23.5		
18–55 years of age	70		0.35–1.15	
♂	63			0.[illegible]0–1.5[illegible]
♀	32			0.[illegible]0–1.4[illegible]

collected under toluene and stored under toluene at 4° C until the determinations were made Blood samples were taken at the beginning of the urine collection period and stored frozen until analysed

All the HP determinations were performed by the method of Prockop and Udenfriend (29) with some practical modifications In the analyses of *urinary total hydroxyproline* the following procedure was used 1 0 ml urine and 1 0 ml concentrated hydrochloric acid were autoclaved in a sealed tube for 3 hours at 124° C The hydrolysis could alternatively be made by treating 1 0 ml urine with 1 0 ml concentrated hydrochloric acid on a 100° C water bath in screw capped tubes of 20 ml capacity (Kimax) The maximal yield was obtained in 12 hours The recoveries in the water bath hydrolysis experiments expressed as average percentages of the recovery values obtained from the samples autoclaved for 3 hours at 124 C were as follows

Time of hydrolysis in hours	3	6	9	12
Percentage recovery (average)	75 4	94 0	96 9	102 5

After the hydrolysis the samples were neutralized with potassium hydroxide and diluted to 15 ml with distilled water When screw-capped tubes of 20 ml capacity were used in the hydrolysis the dilution could be performed in the same tubes The prior precipitation of humin, as suggested in the original method was found to be unnecessary for routine analysis of urine and serum The diluted hydrolysates were filtered and samples taken for the analysis The determinations were continued by procedure II of the original method using half volumes The use of half volumes had the advantage that the method could be carried out in tubes of 20 ml capacity which made it easy to analyse a series of 30—50 tubes at a time The final reading of the optical density was performed 30 minutes after the addition of the colour reagent The samples were analysed in duplicate and reanalysed unless the duplicates did not agree within five per cent

For the assay of *serum free hydroxyproline* the serum was deproteinized with trichloroacetic acid The acid was removed from the clear supernatants with ether Recovery analyses indicated that no losses of hydroxyproline occurred during this preliminary procedure The analyses were carried out by procedure II of the method of Prockop and Udenfriend, using half volumes but 4 ml of toluene instead of 5 ml was used for the final extraction before the colour reaction because of the low levels of free HP in human serum

Results

The values for urinary free and total HP in control subjects are given in table I and for serum free HP in table II The urinary total HP which is the sum of free and peptide bound forms, is expressed both in mg/24 hours and in mg/24 hours/m² body surface area as suggested by Jasin et al (7) Since the HP values seem to be dependent on the age and sex of the subject, the material is divided into three age groups subjects between 17 and 21 years, between 22 and 40 years and over 40 years of age In addition the values for males and females are given separately

The values for the total urinary HP excretion in the groups between 18 and 21 years of age differ markedly in both means and distributions from the values for older subjects among whom the age trend remains insignificant In the values of urinary free and serum free HP no influence of age can be seen The sex difference in urinary total HP excretion expressed as mg 24 hours, and in serum free HP are statistically significant P < 0 05, When however the urinary total HP values are expressed

TABLE IV. Urinary total and free and serum free hydroxyproline values in various disease conditions. Urinary total hydroxyproline is expressed as mg/24 hours/m^2, urinary free hydroxyproline as mg/24 hours and serum free hydroxyproline as μg/ml

Condition	No	Total urinary HP		Free urinary HP		Serum free HP	
Hyperthyroidism[1]	31	23.5–163.5				1.17–3.93	
Hypothyroidism[1]	5	5.2–8.1				0.[illegible]8–0.96	
Acromegaly	2	24.2	44.7	0.88	0.94	–	1.17
Hyperparathyroidism	4	13.7	17.3	0.83	1.32	1.34	1.37
		46.1	218.0	7.20	133.5	–	12.10
Cushing's syndrome	1	10.6		1.35		0.84	
Addison's disease	1	12.7		0.58		1.19	
Turner's syndrome	1	55.4		1.17		1.54	
Klinefelter's syndrome	1	19.2		0.63		–	
Diabetes mellitus	2	9.2	17.3	1.49	1.13	0.91	1.31
Paget's disease of bone	1	35.8		3.64		1.81	
Osteoporosis	2	10.1	25.0	0.37	1.65	1.32	1.80
Cancer with bone metastases	2	44.6	46.6	0.82	0.56	1.79	2.10
Cancer with no bone metastases	1	20.1		0.82		0.87	
Myeloid leukaemia	1	64.6		1.10		2.53	
Systemic lupus erythematosus	3	11.3	12.8	0.62	0.47	0.97,	1.20
		19.0		0.65		–	
Scleroderma	1	16.9		0.40		1.03	
Rheumatoid arthritis	4	13.1	13.5	0.37	0.60	0.80	0.78
		17.7	23.9	0.74	0.43	0.71	1.33
Acute rheumatic fever	2	26.0	33.0	0.74	1.80	1.04	1.55
Polyarteritis nodosa	1	29.4		1.45		1.43	
Marfan's syndrome	2	19.9	36.5	1.17	0.88	1.70	1.24
Malabsorption syndrome	3	40.0	61.9	0.58	1.03	2.04	1.86
		96.0		1.32		2.94	
Myocardial infarction	6	12.8	12.8	0.58	0.80	1.07	1.58
		14.7	15.4	0.76	2.88	0.87	0.97
		21.8	26.9	0.40	0.78	1.56	0.93
Duodenal ulcer	1	14.0		1.19		0.97	
Varicose ulcer of leg	1	15.2		0.95		1.50	
Psoriasis vulgaris	1	19.4		1.37		1.47	
Arteriosclerosis	3	10.1	12.9	0.37	0.64	1.37	0.83
		19.0		0.69		1.18	
Sarcoidosis	3	11.2	12.0	0.89	1.22	0.94	1.41
		18.0		0.63		1.11	
Azotaemia	2	11.1	18.0	2.63	3.56	1.84	2.12

[1] Published in detail elsewhere (15)

as mg/24 hours/m^2, the difference is insignificant, and in the urinary free HP no sex difference can be found. The proportion of free from total urine HP is 2.1 % in the male and 2.6 %, in the female group. In the whole control

group the urinary free HP ranges from 1 2 to 5 2 % of the total HP

Table III gives normal values for urinary free and total HP and serum free HP The values were calculated with the aid of the formula $\bar{x} \pm 2s$ after logarithmic transformation of the HP values The division of urinary total HP values into two groups is due to the higher excretions in young adults compared with the older subjects

Table IV shows the excretion of free and total urinary HP and the values of serum free HP in several disease conditions Increased values were observed in hyperthyroidism in acromegaly, in hyperparathyroidism, in Turner s syndrome, in Paget's disease of bone, in cancer with bone metastases, in myeloid leukaemia, in acute rheumatic fever, in polyarteritis nodosa, in Marfan s syndrome and in the malabsorption syndrome Decreased values were found in hypothyroidism The values were normal in Cushing s disease in Addison s disease in Klinefelter s syndrome, in diabetes mellitus, in osteoporosis, in cancer without bone metastases, in lymphocytic leukemia, in systemic lupus erythematosus, in active scleroderma, in rheumatoid arthritis, in myocardial infarction in duodenal ulcer in varicose ulcer of the leg in psoriasis vulgaris in arteriosclerosis and in sarcoidosis

The hyperparathyroid patients with elevated HP values had greatly altered proportions of free to total HP in the urine The free HP consisted of 35 9 and 10 6 per cent of the total HP In one of these cases which had the highest HP values in the whole study the urinary

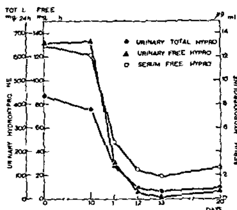


Fig 1 Effect of the removal of parathyroid adenoma on the hydroxyproline values in the urine and serum The operation was performed on the 10th day after two control determinations of hydroxyproline

total and serum free HP were increased about tenfold, whereas urinary free HP was increased over a 100 fold compared with the respective values in the normal subjects In this patient a dramatic fall in all HP values took place after operative removal of a parathyroid adenoma (fig 1)

In the two cases with severe renal azotaemia, high serum free and urinary free HP values were observed in conjunction with a normal level of urinary total HP excretion

Discussion

The results of the present study indicate that HP excretion is dependent on the age sex and body size of the subject The normal urinary total HP is well known from previous studies (2, 6, 7,

TABLE IV Urinary total and free and serum free hydroxyproline values in various disease conditions. Urinary total hydroxyproline is expressed as mg/24 hours/m² urinary free hydroxy proline as mg/24 hours and serum free hydroxyproline as μg/ml

Condition	No	Total urinary HP		Free urinary HP		Serum free HP	
Hyperthyroidism[1]	31	23 5—163 5				1 17— 3 93	
Hypothyroidism[1]	5	5 2— 8 1				0 58— 0 96	
Acromegaly	2	24 2	44 7	0 88	0 94	—	1 17
Hyperparathyroidism	4	13 7	17 3	0 83	1 32	1 34	1 37
		46 1	218 0	7 20	133 5	—	12 10
Cushing s syndrome	1	10 6		1 35		0 84	
Addison s disease	1	12 7		0 58		1 19	
Turner s syndrome	1	55 4		1 17		1 54	
Klinefelter s syndrome	1	19 2		0 69		—	
Diabetes mellitus	2	9 2	17 3	1 49	1 13	0 91	1 34
Paget s disease of bone	1	35 8		3 64		1 81	
Osteoporosis	2	10 1	25 0	0 37	1 65	1 32	1 80
Cancer with bone metastases	2	44 6	46 6	0 82	0 56	1 79	2 10
Cancer with no bone metastases	1	20 1		0 82		0 87	
Myeloid leukaemia	1	64 6		1 10		2 53	
Systemic lupus erythematosus	3	11 3	12 8	0 62	0 47	0 97	1 20
		19 0		0 65		—	
Scleroderma	1	16 9		0 40		1 03	
Rheumatoid arthritis	4	13 1	13 5	0 37	0 60	0 80	0 78
		17 7	23 9	0 74	0 43	0 71	1 33
Acute rheumatic fever	2	26 0	33 0	0 74	1 80	1 04	1 55
Polyarteritis nodosa	1	29 4		1 45		1 43	
Marfan s syndrome	2	19 9	36 5	1 17	0 88	1 0	1 24
Malabsorption syndrome	3	40 0	61 9	0 58	1.03	2 04	1 86
		96 0		1 32		2 94	
Myocardial infarction	6	12 8	12 8	0 58	0 80	1 07	1 58
		14 7	15 4	0 76	2 88	0 82	0 92
		21 8	26 9	0 40	0 78	1 56	0 93
Duodenal ulcer	1	14 0		1 19		0 97	
Varicose ulcer of leg	1	15 2		0 95		1 50	
Psoriasis vulgaris	1	19 4		1 37		1 47	
Arteriosclerosis	3	10 1	12 9	0 37	0 64	1 32	0 83
		19 0		0 69		1 18	
Sarcoidosis	3	11 2	12 0	0 89	1 22	0 94	1 41
		18 0		0 63		1 11	
Azotaemia	2	11 1	18 0	2 63	3 56	1 84	2 12

[1] Published in detail elsewhere (15)

as mg/24 hours/m², the difference is insignificant, and in the urinary free HP no sex difference can be found The proportion of free from total urine HP is 2 1 % in the male and 2 6 % in the female group In the whole control

synthesis (9, 19) or degradation of mature collagen secondary to mobilization of bone mineral (20) The rapid fall in the HP values found after operation and the well known increased bone formation and decreased lysis in hyperparathyroid patients after successful operations suggests that the hyperparathyroid effect is mainly due to degradation of mature collagen and not to increased collagen synthesis in osteitis fibrosa as has been postulated (19)

Although *cortisone* has an inhibitory effect on the formation of collagen the administration of cortisone to human subjects and adults rats (16, 34) has not been observed to influence HP excretion Neither have abnormal values been observed in Addison s or Cushing s disease In young rats, however cortisone greatly reduces the excretion of HP in the urine, because of the reduced proportion of urinary HP derived from the newly synthesized collagen fractions (13, 14) Whether the low normal values in some patients with Cushing s disease reported in this study and by Klein et al (23) could also be due to reduced synthesis of collagen cannot be stated, because of the small amount of relevant data reported for this disease

In accordance with the findings of Benoit et al (2), both elevated and normal values were observed both in *Turner s syndrome* and in *Klinefelter s syndrome* with high urinary pituitary gonadotropins As suggested by Benoit et al the elevated HP values are more probably due to delayed maturity than to a direct effect of pituitary gonadotropins

Increased urinary excretion of HP has been observed in this study and other reports in several *diseases affecting bone*, such as Paget s disease (2, 4, 6), fibrous dysplasia of bone (6), cancer with bone metastases (23, 26) and rickets (21) The HP values in osteoporosis may be normal or possibly slightly elevated (23, 24) The greatly increased value in one case of myeloid leukaemia in this study compared with the normal excretion in lymphatic leukaemia may be indicative of bone affection in the former disease The above examples support the suggestion of Klein et al (19) and Dull and Henneman (4) that changes in bone collagen alone can greatly alter the HP excretion values However, it seems apparent that altered HP values caused by some hormonal actions as by growth hormone thyroid hormone and cortisone are reflections of changes in the body collagen as a whole In addition there are a number of conditions, such as post partum involution of the uterus (18) and extensive burns (22), in which large extraosseous changes in collagen cause increased values of HP By contrast, in small extraosseous connective tissue forming processes, as in patients with myocardial infarction duodenal ulcer and varicose ulcer of the leg no change in the excretion of HP can be found

Patients with *connective tissue disorders* have shown normal or slightly elevated urinary HP excretion (34) The present results agree with these observations Significantly increased values have been reported only in active scleroderma (30) We and several other workers have not been able to confirm this

finding (cf 33) Nevertheless, the significantly elevated excretion in 2 cases of acute rheumatic fever and one case with polyarteritis nodosa in this study *indicates that probably some of the* collagen diseases might be accompanied by moderately altered values in the active phase of the disease

HP excretion is elevated in the majority of the tested cases of Marfan's syndrome (8, 32) The determination of HP might be of value if there are diagnostic difficulties in this disease

The high HP values in the *malabsorption syndrome* in the two young women might be due to the delayed maturity secondary to malabsorption However, elevated values were also found in an old woman Further, altered values in patients with tropical sprue have been reported (31) Therefore an elevating mechanism primarily caused by these gastrointestinal disturbances cannot be excluded It is possible that changes in the calcium metabolism of the patients with malabsorption are the explanation of the high values

The normal HP values found in patients with psoriasis vulgaris, arteriosclerosis and sarcoidosis indicate that, if present, the change in collagen metabolism in these diseases is too small or slow to alter the HP values significantly

In the two cases with azotaemia, urinary and serum free HP were markedly increased as compared with the urinary total HP values, which were within the normal range Finlayson et al (5) have reported lowered values of urinary total HP excretion in five patients with acidotic uraemia These findings could be interpreted as evidence of an increased degradation of peptide bound HP to free HP in azotaemia

The parallelism in the values of urinary and serum HP suggests that the changes in these values are in general due to altered collagen metabolism and not to renal changes In normal subjects urinary free HP like any free amino acid, is reabsorbed almost completely in the renal tubules The greatly altered proportion of free to total HP in the urine of two patients with hyperparathyroidism indicates, however, that there may be a defect in the reabsorption mechanism of free HP in these cases Excretion of peptide-bound HP, which has been observed to be secreted by the renal tubules in loading tests (3), has not been proved to be altered on the renal level in any disease condition

Conclusions

The hydroxyproline values have been observed to have a relatively constant level and small variation in normal subjects and patients with several diseases However, the characteristic changes in some diseases suggest that the determination of urinary total hydroxyproline and serum free hydroxyproline may be of value in the following cases

1 In diagnosis and treatment of thyroid diseases As a diagnostic aid the method is especially useful in cases where the determination of protein bound iodine is not possible The effect of therapy is rapidly reflected in the hydroxyproline values

2 As an index of response to therapy in growth hormone treatment and as an indicator of activity of acromegaly

3 As a diagnostic aid in Marfan s syndrome

4 When evaluating the extent of bone manifestations in various diseases

Summary

The values of urinary free and peptide bound hydroxyproline were measured in 72 control subjects and 89 patients with various diseases and the values of serum free hydroxyproline in 95 control subjects and in 86 patients Practical improvements in the methods of hydroxyproline determination are suggested Three factors age sex and body size, were found to have an influence on the hydroxyproline values in the control subjects For clinical purposes the following normal values are suggested urinary total hydroxyproline for persons between 18 and 21 years of age 13 0—28 0 mg/24 hours/m² and for persons between 22—55 years of age 8 5—23 5 mg/24 hours/m serum free hydroxyproline for males 0 70—1 55 μg/ml and for females 0 70—1 40 μg/ml Increased values were found in hyperthyroidism in acromegaly in Turner s syndrome in hyperparathyroidism and in some other diseases in which there were bone changes in acute rheumatic fever in polyarteritis nodosa in malabsorption syndrome and in Marfan s syndrome Free hydroxyproline was greatly increased compared with total hydroxyproline in the urine of two patients with hyperparathyroidism and in two patients with azotaemia The determination of total hydroxyproline in the urine and of free hydroxyproline in the serum is considered to be of value in the diagnosis of thyroid diseases and Marfan s syndrome in following the response to therapy in thyroid diseases acromegaly and growth hormone treatment and when evaluating the extent of pathological alterations in various diseases affecting the bones

Acknowledgement

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Addendum

The hyperparathyroid pat ent presented in fig 1 had during the next months after the operat on st ll symptoms ind cative of hyperparathyro d sm although the serum and urinary calcium and phosphorus levels were normal Urinary total HP values on the other hand began to rise and then rema ned at a level of 132 to 166 mg/24 h On the bas s of this find ng and on clinical grounds a second explorat on was performed nine months after the first operat on Add tional parathyro d adenoma tissue was found and removed Thereafter a pronounced cl nical improvement occurred and the ur nary HP excre t on has returned to normal level Thus in this case the HP analysis was found to be of decis ve d agnost c importance

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Intracardiac Phonocardiography in the Diagnosis of Small Isolated Ventricular Septal Defects

By

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The accurate diagnosis of a small ventricular septal defect is now possible with more sensitive methods for detecting small left to right shunts than the usual oxygen determinations during right heart catheterization

It is the aim of this paper to show that intracardiac phonocardiography during a simple right heart catheterization may be used in the diagnosis and may contribute to the understanding of the auscultatory findings in patients with small ventricular septal defects

Methods

The Allard Laurens micromanometer an inductance type transducer was used to record pressures and intracardiac phonocardiogram during a right heart catheterization (9) The micromanometer is mounted on the tip of a no 8 double lumen catheter one lumen — through which pressure was measured and blood was sampled for oxygen determination — opens 1 5 cm from the tip the other lumen carries the wires to the micromanometer

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The transducer consists of an inductance coil with a core inside which is attached between two membranes when the distal membrane is set into vibrations the core is moved axially in the coil thus causing inductance changes which frequency modulate an oscillator of 150 kc The frequency changes are transformed into voltage changes and through filtration the low frequency vibrations and high frequency vibrations are separated as pressure waves and cardiac sounds respectively

The amplitude of the heart sounds and murmurs is measured and compared by a calibration signal delivered by the electronic unit as a vibration corresponding to pressure variations of 1 mm of mercury

Diagnosis

During systole a jet of blood passes from the left to the right ventricle through the ventricular septal defect and a rather high pitched systolic murmur is produced in the defect and transmitted with the blood flow When this murmur is recorded by the micromanometer placed in the right ventricle the diagnosis is established (fig 1) (4 8

The murmur is most intense and of highest frequency when the tip of the catheter is situated in the jet at the defect and it is



Fig 1 Intracardiac phonocardiogram (upper tracing) showing holosystolic murmur in the outflow tract of the right ventricle (case no 7492) In the upper right margin the calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line Pressure is recorded both with the micromanometer at the tip of the catheter (middle tracing) synchronously with the sound and through the sidehole (lower tracing) 1 5 cm from the tip the latter tracing being calibrated to 0 and 25 mm Hg

fainter as the tip is moved away from the point of origin thus it is possible not only to diagnose but also grossly to localize the site of the defect by watching the tip of the catheter during fluoroscopy when the murmur has its maximum intensity

The murmur due to a left to-right shunt of sufficient magnitude to be measured by oxygen determination is usually present in all parts of the right ventricle A murmur due to a smaller defect — that is with a left to-right shunt of 20 % or less is often only present near the defect all parts of the right ventricle have to be carefully explored with the catheter tip until the murmur is found

In patients with infundibular pulmonary stenosis a systolic murmur is present in the right ventricle (8) by pressure measurements during the right heart catheterization this condition may easily be ruled out

The systolic murmur of aortic stenosis may sometimes be registered in the right ventricle (7) as it also is transmitted to the superior caval vein and/or to the right atrium it is easily distinguished from the murmur of ventricular septal defect

Materials and results

From March 1964 to May 1965 the diagnosis of ventricular septal defect was established in 13 patients by intracardiac phonocardiography during right heart catheterization where the oxygen determinations failed to demonstrate any left to right shunt

The presence of a shunt was confirmed with the platinum electrode catheter with hydrogen inhalation (2) in nine of the patients (table I)

In addition to the systolic murmur recorded in the right ventricle a fainter systolic murmur was found in the pulmonary artery in 10 patients No other murmurs were recorded in the right side of the heart The pressures were normal in all patients (table I)

None of the patients had definite cardiac symptoms The physical findings and roentgenogram of the chest in all were within normal limits except for the systolic murmur Electrocardiogram showed incomplete right bundle branch block in one patient (case no 4051) and intermittent right bundle branch block in another (case no 6401) while it was normal in the remaining patients

TABLE I Findings in 13 patients with small ventricular septal defects

			High pitched systolic murmur						Systolic pressure in			
Case no	Age (yrs)	Thrill	Maximum left ic space	Grade 1—6	Length systolic	Form (figs 2—8)	Slightly harsh	H used	Pulm art (mm Hg)	Outfl r v (mm Hg)	Infl r v (mm Hg)	Site (fig 9)
6 526	28	+	3 rd	4	holo-	5	+		22	22	24	1—2
7 238	10		3 rd	3	protomeso	8			18	18	22	1 2
7 492	11		3 rd	2	holo-	3		+	16	17	19	1—2
7 243	17		4 th	3	holo-	3		+	21	21	23	1—2
7 4 6	22	+	2 nd	4	holo-	6	+	+	26	26	28	1 2
4 051	20		3 rd	2	holo-	2		+	20	20	26	3
6 401	36		4 th	2	holo-	3		+	22	—	26	3
7 403	14	+	3 rd	4	holo-	6	+	+	23	23	30	1 2
5 707	28		3 rd	3	proto-	7		+	24	24	25	3
7 466	15	+	3 rd	3	holo-	4		+	27	27	29	1 2
6 434	26		4 th	2	proto-	7		+	17	17	22	4
7 558	27		4 th	3	holo-	3			19	19	22	1—2
5 526	21		4 th	2	holo-	2			25	25	30	1 2

Left ic space = left intercostal space   H = hydrogen electrode   Pulm art pulmonary artery
Outfl r v outflow tract of the right ventricle   Infl r v inflow tract of the right ventricle

Auscultatory findings

In all patients the murmur was fine high pitched grade 2—4 (of 6) having some harsh components only in three patients (table I) It was holosystolic in 10 patients the form varying from plateau to crescendo and ejection form with peak intensity in early mid or late systole (figs 2—6)

Three patients had a murmur which occupied the first 1/2 or 2/3 of systole (figs 7—8) in two of these patients (cases no 5707 and 6434) there was a short interval between the first heart sound and the onset of the murmur (fig 7)



Fig 2 External phonocardiogram in case no 4051 The murmur is holosystolic and plateau shaped A three-channel ink jet recorder (Mingograf 31 B Elema Schonander) was used the upper channel recording the electrocardiogram and the two other channels recording the phonocardiogram in the 100 hz and 400 hz range respectively



Fig 3 External phonocardiogram in case no 7243 The murmur is holosystolic with late crescendo form

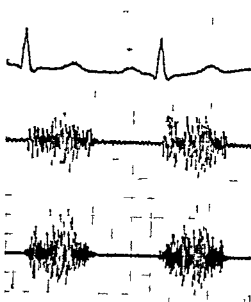


Fig 5 External phonocardiogram in case no 6526 The murmur is holosystolic of ejection form with mid systolic peak intensity

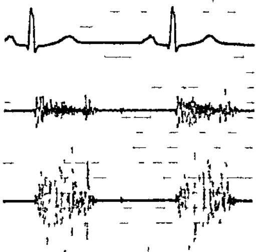


Fig 4 External phonocardiogram in case no 7466 The murmur is holosystolic of ejection form with peak intensity in early systole



Fig 6 External phonocardiogram in case no 7476 The murmur is holosystolic of ejection form with peak intensity in late systole

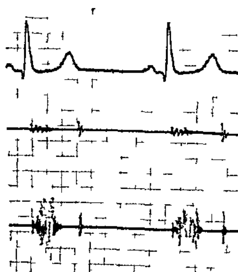


Fig 7 External phonocardiogram in case no 5707 The murmur is protosystolic of ejection form there is a short interval between the first heart sound and the onset of the murmur



Fig 9 The ventricular septal wall seen from the right ventricle showing the different sites of defects 1 outflow tract defect immediately beneath the pulmonary valve and superior to the crista supraventricularis 2 outflow tract defect inferior to the crista supraventricularis (most common site) 3 inflow tract defect beneath septal leaflet of tricuspid valve 4 inflow tract defect near apex At 1 3 and 4 the defect is in the muscular septum at 2 in the membranous septum (From Kirklin et al The journal of thoracic and cardiovascular surgery *33* 45 1957 C V Mosby Co St Louis Missouri)

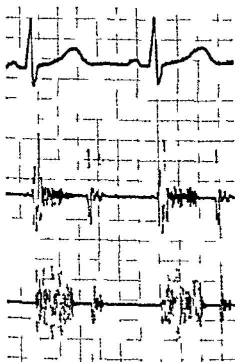


Fig 8 External phonocardiogram in case no 7238 The murmur is protomesosystolic of ejection form beginning with the first heart sound

Localization of the defect

Of the 10 patients with a holosystolic murmur the defect was localized to the outflow tract in eight patients (fig 1) to the area just within the tricuspid valves in one patient and to the transition betweeen the inflow and the outflow tract near the left border of the heart in one patient (fig 9 table I)

Of the three patients with a shorter murmur the defect was localized to the outflow tract to the inflow tract near the left border of the heart (fig 10)

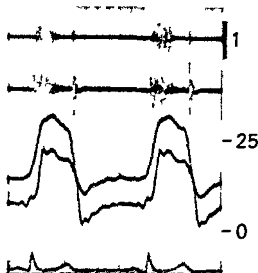


Fig 10 Intracardiac and external phonocardiogram (second tracing from above) registered simultaneously in case no 5707 The micromanometer was in the inflow tract of the right ventricle.

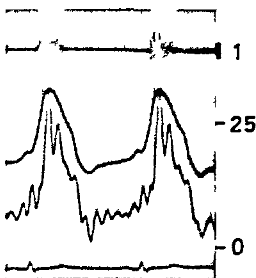


Fig 11 Intracardiac phonocardiogram in case no 6434 The micromanometer was in the apex of the right ventricle.

and to the apex region (fig 11), respectively

While it was not possible to determine whether the defects in the outflow tract were in the membranous or the muscular septum, it is obvious that the defects in the inflow tract all must be in the muscular septum

Discussion

The accurate diagnosis of a small ventricular septal defect has some importance, not only because of the differentiation from the "functional" systolic murmur (10, 12), but also because of the risk of bacterial endocarditis (1)

Often it is possible to make the diagnosis clinically, and the characteristics of the physical findings of small defects have been described by several other authors (6, 10, 11, 12)

But cases remain with a doubtful clinical diagnosis, and when hemodynamic studies are performed, the methods have to be refined to avoid unnecessary extensive investigations in a fruitless search for the diagnosis

By intracardiac phonocardiography the diagnosis may be made during a simple right heart catheterization. In addition, a localization of the defect is possible

It has been suggested, that defects associated with short, ejection type murmurs are located in the muscular septum, and that the contraction of the septum during the last part of systole closes the defect which would account for the characteristics of the murmur (3 5, 10) Of three patients with a short systolic murmur investigated by me the defect was clearly localized to the muscular septum in two patients, while in the third patient the site in the outflow tract did not permit of distinction between membranous and muscular defect.

Summary

The diagnosis and localization of small ventricular septal defects is possible by intracardiac phonocardiography during a simple right heart catheterization

The diagnosis was established in 13 patients in whom oxygen determinations failed to show any left to-right shunt In nine of the patients the shunt was confirmed with the hydrogen electrode

Three patients had a short systolic murmur, in two of these patients the defect was localized to the muscular septal wall, while the distinction between membraneous and muscular septal defect was not possible in the third patient

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9-Alpha-fluorohydrocortisone in the Treatment of Postural Hypotension

By

M H Frick

After pioneer animal studies (1, 3) the enhanced potency of halogenated steroids in particular 9 alpha fluorohyd rocortisone, was demonstrated in man by Liddle et al (9) Subsequent reports have confirmed the effectiveness of this 9 alpha halogenated hydrocortisone in various clinical conditions (4 6, 8)

The fact that 9 alpha fluorohydrocor tisone can raise blood pressure was pointed out by Owen et al (11), who observed that hypertension developed in an infant with adrenogenitalism and in three patients of six with Addison s disease treated with this drug The observation revealed that the drug is to be used with caution but it also disclosed a new steroid to be used in the treatment of hypotensive states After the first report of a successful treatment of postur al hypotension with 9 alpha fluorohyd rocortisone (7) twelve more patients so treated have been reported (13, 14)

The present report describes the experience gained in treating three patients with postural hypotension with 9 alpha fluorohydrocortisone The diag nosis was based on a postural fall in both the systolic and the diastolic blood pressure and a fixed heart rate with or without other signs of autonomic nervous system imbalance

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Case reports

Case 1 A 60-year-old tailor who had consum ed one bottle of distilled beverages a week for the last 40 years Since 1956 he had occasionally suffered from heart palpitations and dyspnea in connection with muscular effort These symptoms gradually became worse and were for the last three years associated with dizziness The vertigo occur red both in changing from recumbency to standing and during muscular exertion The situation was worse in the morning and the patient often had to roll himself out of bed On October 10 1963 the patient was unable to rise out of bed because of heart palpitations and vertigo and was admitted to the hospital

On admission the general impression was commensurate with the history of 40 years drinking Spider nevi and palmar erythemas

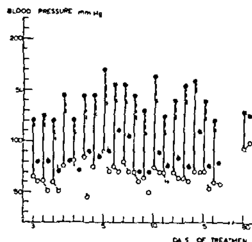


Fig 1 Blood pressure response to therapy The dotted lines indicate the change caused by standing

were illustrative The liver extended to 4 cm below the costal margin and was not tender on pressure No heart murmurs were heard but some moist rales were audible in the base of the left lung B P was 115/75 when the patient was supine The heart rate was regular at 160 beats/min The pulse deficit was 30 beats/min The deep tendon abdominal and cremaster reflexes were normal and symmetrical The left pupil was elliptic and smaller than the right A fine tremor was observed in both hands The ECG revealed atrial flutter with 2 1 block Chest X ray showed a heart of 680 ml/M² BSA emphysema of the lungs a sclerotic aorta and a retrosternal goiter

The patient was kept in bed and digitalized The rhythm was converted with chinidin Laboratory data obtained during this period showed BSR 16 mm/1 h Hb 16 4 g/100 ml packed cell volume 46 % leukocytes 8 400/mm³ no protein sugar or formed elements in the urine Ehrlich — Schlesinger + Harrison — Meulengracht 1 3 TT 56 % GOT 21 units bromsulfonphthalein retention 7 % in 33 min BMR ± 0 % serum cholesterol 168 mg % and PBI 5 0 μ % plasma creatinine 0 80 mg % normal acid base balance and serum electrolytes LE cell phenomenon and nuclear antibody reactions as well as blood Wassermann reactions negative urinary excretion of catecholamines 30 μg/24 h and of vanillin mandelic acid 1 9 mg/24 h, cerebrospinal fluid was under normal pressure Nonne — Pandy — 3 cells/mm³ Wassermann reactions negative and mastix normal

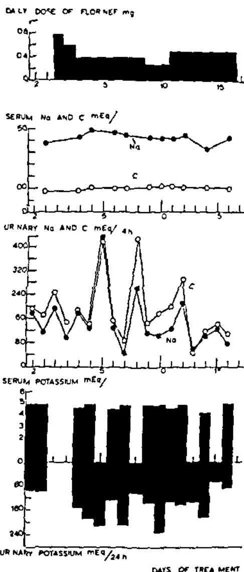


Fig 2 Serial readings of serum and urinary electrolytes during 9 alpha fluorohydrocortisone therapy

After the restoration of the sinus rhythm the postural blood pressure reactions were examined with the following results B P

supine 120/65 standing 80/60, pulse rate 40 beats/min in both positions The treatment was begun with 25 mg of Neo-Synephrine® in the morning with some relief of dizziness This was however considerable and the patient was put under a 9 alpha fluorohydrocortisone regimen (Florinef®) The other daily drugs including 0 725 mg of Digoxin, 1 0 g of chinidin sulf, 52 mEq of potassium and 25 mg of Neo-Synephrine were kept unchanged The patient was on an ordinary hospital diet The blood pressure response is illustrated by fig 1, and the behavior of the electrolytes by fig 2 The pulse rate was fixed throughout the treatment The hematocrit steady declined from 48 5 to 41 0 vol %

The patient was discharged from the hospital in good condition with a daily dose of 0 5 mg of Florinef in addition to potassium chinidin and Digoxin The last control was 6 months after he had left the hospital The blood pressure response to standing was normal (fig 1) The patient was then under the influence of liquor which may have affected the blood pressure response Later on he was lost from the follow up

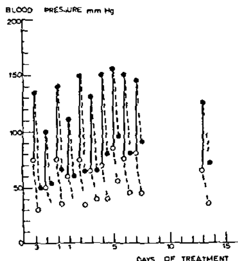


Fig 3 Blood pressure response to 9 alpha fluorohydrocortisone therapy

Case 2 An 80 year-old woman who had suffered from severe dizziness since 1958 The history revealed the following 1916 pulmonary tuberculosis 1936 idiopathic hypothyroidism obstipation since 1958 strong normochromic anemia since 1958 several X ray studies of the gastro-intestinal tract with a suspicion of ventricular carcinoma 1961 unproved suspicion of a renal tumor and a strong anemia and later in the same year removal of a renal cyst 1963 hospitalized because of severe vertigo and anemia which was attributed to renal failure evoked by a chronic pyelonephritis

The patient was admitted to the hospital on April 8 1964 because of marked dizziness making her bedridden and resistant to the treatment hitherto given Digoxin ephedrine Ritalin® and Priscophen®

Physical examination revealed a coarse tremor in both hands normal abdominal and deep tendon reflexes a palpable mass *in the abdomen and nothing special in the* heart and the lungs The decline of both the systolic and the diastolic blood pressure on changing from recumbency to standing was marked The pulse rate was invariable Because of dizziness the patient was unable to stand without help and often fainted even when supported Chest X ray taken with the patient supine revealed emphysema sclerotic aorta and left ventricular prominence ECG showed S T segment depression in left ventricular precordial leads Laboratory data BSR 25 mm/1 h Hb 9 1 g/100 ml packed cell volume 29 %, leukocytes 5 000/mm³ with roughly normal differentiation no protein sugar or formed elements in the urine plasma creatinine 1 52 mg % normal acid base balance GOT 10 units LDH 310 units LE cell phenomenon negative nuclear antibody test negative serum iron 98 μ % Schilling test 4 5 % uropepsin excretion 14 9 units bone marrow smear revealed normal morphology plasma volume measured with Evans blue 2 627 ml calculated red cell volume 1 126 ml and total blood volume 3 753 ml *paper electrophoresis of the serum* proteins was normal serum PBI was 4 7 μ%

Because of the patient s poor condition the abdominal mass was not closer examined

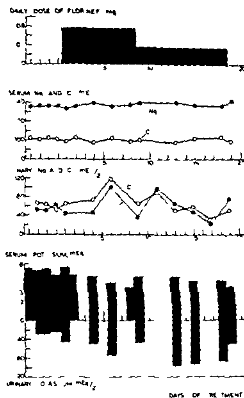


Fig 4 Serum and urinary electrolytes during 9 alpha fluorohydrocortisone therapy Normal hospital diet

A general supportive therapy consisting of elastic stockings and corsets was of no effect on the postural blood pressure reaction Treatment with 9 alpha fluorohydrocortisone was begun with some relief in the symptoms The blood pressure response was rather poor however (fig 3) The pulse rate remained fixed on changing position The hematocrit was unchanged and a further plasma volume measurement after 14 days treatment showed a plasma volume of 2 287 ml and a red cell volume of 980 ml The electrolyte response to the treatment is illustrated by fig 4

No decisive improvement was achieved and the patient was discharged from hospital after one month s trial Later on in a private Home for the Aged a 2 week s trial with 2 mg of Florinef per day was made This raised the supine B P to 180/110 but a postural drop of 80/50 resulted in syncopes and sometimes convulsions and the treatment was stopped

Case 3 A 66-year-old teacher, female, had suffered from bronchial asthma between the ages of 25 and 55 years A period of two years before admission was characterized by a progressive lightheadedness and vertigo leading ultimately to daily syncopes sometimes accompanied by convulsions

The patient was admitted January 27 1965 She was in good general condition The deep tendon reflexes were normal and symmetrical, the Babinski sign was negative on both sides The pupils were larger than normal and reacted poorly to light The right eye had a weakened convergent activity A grade 2/6 systolic murmur with no diagnostic characteristics was audible on the left sternal border Some rales were audible in both lungs No peripheral edema or liver enlargement were found A marked decline occurred in both the systolic and the diastolic blood pressure in changing from recumbency to standing The pulse rate was fixed The eye grounds were of grade II of Keith Wagener

Chest X ray revealed a heart of normal configuration but of a volume of 930 ml (530 ml/M² BSA) exceeding the normal There were no signs of heart failure or pulmonary pathology The skull and sella turcica were normal in X ray ECG was normal

Laboratory data BSR 8 mm/1 h Hb 10 3 g/100 ml packed cell volume 33 %, leukocytes 7 600/mm³ with a shift to the right in the differentiation no protein sugar or formed elements in the urine low specific gravity of the morning urine and endogenous creatinine clearance 70 ml/min normal bilirubins serum cholesterol 218 mg % and PBI 5 3 μ %, a normal Schilling test normal serum electrolytes with a potassium value of 5 2 mEq/l around the upper limit of normal LE cell phenomenon and nuclear antibody test negative, normal blood sugar iron and TIBC, the 24 hour excretion of catecholamines 45 μg and vanillin mandelic acid 3 0 mg basal 17 OH-corticoid and 17

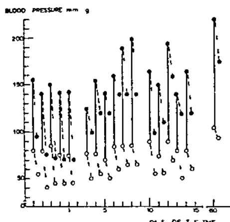


Fig 5 Blood pressure response to therapy The postural drop as indicated by the dotted lines is clearly less marked

ketosteroid excretions were normal for the age and were adequately augmented by ACTH The same was true of plasma corticoids A Metopiron infusion test gave no diagnostic help

The postural blood pressure reaction was little influenced by oral Neo-Synephrine® and elastic supports A 9 alpha fluorohydrocortisone treatment gave a good response as is illustrated by fig 5 The electrolyte balance was characterized by potassium depletion necessitating supplementation (fig 6) A control chest X ray taken because of a mild respiratory infection revealed a clearly increased heart volume averaging 1 150 ml The hematocrit steadily declined from 33 to 28 vol %

The patient was discharged symptom free with a daily dose of 0 5 mg of Florinef after one month s treatment She was controlled 6 weeks thereafter in the out patient department The blood pressure decline from supine to standing was modest (fig 5) The heart rate was fixed The serum potassium was 3 8 mEq/l

Discussion

Efforts to treat postural hypotension with pressor amines have in general not been successful (2, 7) although pathogenetically implicated (10) The use of desoxycorticosterone acetate has in some cases resulted in marked improvement (5) but failures have also been observed (13)

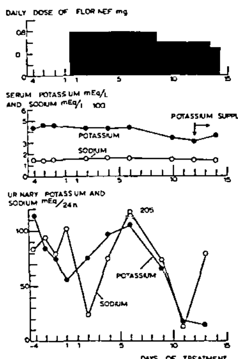


Fig 6 Serum and urinary potassium and sodium during the treatment Normal hospital diet

Amongst the thirteen patients so far reported 9 alpha fluorohydrocortisone has resulted in moderate or marked improvement in eleven cases In the six patients for whom there were detailed blood pressure data (7 14) the response has been a pure elevation of the blood pressure level in three patients and both a basic elevation and a decline in the postural drop in another three All the present patients showed this combined effect (figs 1 3 and 5) While the response entailed benefit for two patients the second case could

not be mobilized This patient demonstrates that the induced elevation of the blood pressure is of no help in alleviating the symptoms if the cerebral circulation does not tolerate the postural blood pressure drop inherent in this disease

Cases 1 and 3 showed signs of enhanced plasma volume, i e , a decline in the hematocrit and an increase in the heart volume The elevation of the blood pressure in these patients thus supports the generally accepted view that the blood pressure raising effect of 9 alpha-fluorohydrocortisone is mediated via an augmented plasma volume The basic defect in the release of norepinephrine in the sympathetic nerve endings has been shown to be unaltered by 9 alpha-fluorohydrocortisone treatment (7) Case 2 was exceptional in showing a decline in the plasma volume during the treatment This decrement, probably due to renal factors, was nevertheless associated with a rise in the blood pressure, a fact which is pertinent to the observations of Owen et al (11) that hypertension developed in three of their patients without any signs of edema The findings of Raab et al (12) that desoxycorticosterone acetate potentiates the effects of norepinephrine may be re emphasized

The occasional determinations of the serum and urinary electrolytes in the present cases show within the variations caused by an unbalanced hospital diet an increased potassium excretion and a decline in the serum potassium concentration The changes in serum and urinary sodium and chlorides were more inconsistent, but the rather steady serum sodium concentration combined with the signs of elevated plasma volume in cases 1 and 3 suggest sodium retention These findings are consistent with the sodium retaining and potassium excreting properties of 9 alpha fluoro hydrocortisone found in cases of endocrine disease (4, 6) and of postural hypotension (14)

This sodium retaining activity of 9 alpha fluorohydrocortisone, in addition to the effect on the extracellular fluid volume, may offer an explanation for the blood pressure rise in terms of stimulation of the release of a vasopressor factor from the posterior pituitary This sequence of events is relevant in the light of the observation that, unlike normal subjects patients with postural hypotension exhibit a pressor response to ADH (7, 15) The increased potassium excretion evoked by the drug calls for action in the form of a close follow up and of potassium supplementation at the right time

This series, as for the previous ones, shows that 9 alpha fluorohydrocortisone is a potent drug and apparently the best now available for the treatment of postural hypotension It also shows that therapeutic failures unavoidably occur in patients, presumably old and sclerotic individuals, who in spite of the elevation of the blood pressure do not tolerate the postural blood pressure fall Complete cure of this disease would require a drug affecting the primary neurogenic fault, a hope that awaits realization

Summary

Three patients with postural hypotension were treated with 9 alpha fluoro-

hydrocortisone The supine and standing blood pressures were elevated in all three The postural blood pressure drop was also decreased and resulted in marked improvement of the dizziness and the syncopal trend in two patients One patient, an 80 year-old woman, remained bedridden because of severe postural symptoms In this patient the blood pressure was elevated in spite of a fall in plasma and blood volumes due to long standing renal anemia In the other two patients the blood pressure rise was accompanied by signs suggestive of an increased plasma volume

Brief periods of serum and urinary electrolyte measurements revealed increased potassium excretions Potassium supplementation was given to two patients Data on sodium and chlorides were suggestive of sodium retention

Acknowledgement

Some samples of Florinef® were provided by E R Squibb & Sons

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Uro-genital Infection of the Male in Relation to Ankylosing Spondylitis and Rheumatoid Arthritis

By

HELJO JULKUNEN, KARI PIETILA and JAAKKO ELO

It is known that genito urinary infection may be of significance in the etiology of ankylosing spondylitis Fifteen per cent of the patients of Forestier et al (3) had had gonorrhea According to two other papers most cases had chronic prostatovesiculitis (9 10) In a further study 89 per cent of cases of ankylosing spondylitis and 50 per cent of patients with rheumatoid arthritis had prostatovesiculitis (5)

Grimble (4) has shown that in all his eight cases of ankylosing spondylitis in two of 18 cases of rheumatoid arthritis and in eight out of 9 cases of prostatitis there were antibodies to prostatic antigen in the serum The existence of auto immune' prostatitis is possible

The present follow up study has been made to find out how often ankylosing spondylitis and rheumatoid arthritis occur in a group of non selected patients with uro genital infection

Material

The material consists of 149 patients who had been admitted to the urologic department for treatment of acute or chronic prostatovesiculitis and non specific epididymitis during the years 1951—1963 104 patients (70 per cent) came to the present follow up study Among these 104 patients 77 had prostatovesiculitis and 27 epididymitis The mean age of the patients was 46 years and the mean age at onset of the urologic complaint was 35 years The mean duration of the follow up period was 7 5 years in both groups

The diagnosis of prostatovesiculitis was based on the history symptoms and clinical examination The clinical examination consisted of rectal palpation In one part of the material also a microscopic examination of the expressed prostatic fluid was performed

In the follow up examination of the sacroiliacal joints the lumbar and lower thoracic spine were roentgenographed The sacroiliac joints were roentgenographed in antero-posterior and postero-anterior the spine in antero-posterior and lateral projections according to Romanus and Yden (11) In connection with the examination the data concerning rheumatic symptoms of the spine and joints were added to the history In 38 cases there were joint symptoms and a roentgenologic study of the diseased joints was performed

The control group consisted of 100 male patients of the Second Medical Clinic Their mean age was 51 years In none of them where there present either rheumatic

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TABLE I The main results of the clinical and roentgenological follow up study of 104 cases of urologic material and 100 control cases collected for roentgenological study of the spine

	Urologic material (104 cases)	Control material (100 cases)
Ankylosing spondylitis	4	—
Rheumatoid arthritis	1	—
Degenerative spinal disease	44	54
Reactive sacro-iliacal changes	3	2
Syndesmophyte like changes with spondylosis	2	3
Anomalies of the spine	12	8
Degenerative joint disease	4	—

joint complaints or genito-urologic symptoms or signs In each case a roentgenologic study of the spine and sacro-iliac joints was performed The control patients were admitted to the hospital because of gastro-intestinal disease in 14, cardiovascular disease in 20 pulmonary disease in 20, endocrine or metabolic disease in 21, blood dyscrasias in 17 and other diseases in 8 cases

Results

The main results are shown in table I

Ankylosing spondylitis was verified in four cases in the follow up study Their case histories are given in brief

Case 1 V R 36-year-old stock keeper At 28 years he had discharge from the urethra. At 29 swelling in right ankle knee and in little finger during 6 months Treated in rheumatologic clinic At 31 again discharge and heel pains during 3 months At 32 urologic complaint Chronic prostatovesiculitis was diagnosed in the urologic clinic At the same time nocturnal pains in the lower spine later in the thoracic spine In the follow up study in 1965 normal joint status was noted, also in the roentgenologic study Stiffness was found in the thoracic spine Inspirium-expirium difference was 4 5 cm. Bilateral sacroiliitis (erosions and sclerosis) and syndesmophytes in the thoracic spine were found Changes typical for chronic prostatovesiculitis were found on rectal palpation The first degree a v block was noted in ECG ESR 5 mm/h Waaler Rose titer 90 Latex —

Case 2 T K. 54 year old typographer At 37 pains in both ankles and lower spine At 42 swelling in ankles and pain in the heels At 43 urologic symptoms first observed At 49 in the urologic clinic prostatovesiculitis was verified At 51 swelling and stiffness in right ankle, left knee and pains in the whole spine In the rheumatologic clinic a negative Latex test was noted and ESR was 100 In the follow up study in 1965 stiffness in subtalo-joint, heel pains and stiffness in thoracic spine were noted Inspirium expirium difference was 4 cm There were changes typical for chronic prostatitis In the roentgenologic study of the spine a unilateral sacro-iliitis syndesmophytes in lumbar and thoracic spine and ankylosis in the sterno-manubrial synchondrosis were noted The roentgenologic study of the ankles and feet showed osteoporosis and heel spurs ESR 26 mm/h Latex —

Case 3 J S 31 year old clergyman At 15 arthritis in the ankles shoulders knees, feet and pains in the heels and sternoclavicular joints Treated in the rheumatologic clinic At 25 together with the urologic complaint nocturnal pains in the lower spine In the urologic clinic a chronic prostatitis was noted In the follow up study in 1965 stiffness in the lumbar spine

and pains in metatarsophalangeal joints were noted In a roentgenologic study bilateral ankylosis of sacroiliac joints and squaring of the thoracic vertebrae were noted Also destructive changes in metatar sophalangeal joints and great calcanear spurs were found Inspirium expirium difference was 4 5 cm There were changes characteristic of chronic prostatovesiculitis ESR 41 mm/h Waaler Rose titer 0

Case 4 J L 64-year old plasterer Gonorrhea at 21 At 35 he had pains in the lower spine heels and toes During last five years continued pains in the whole spine and swelling in the right wrist At 55 he came to the urologic clinic because of acute urologic complaint Chronic prostatovesiculitis was found No rheumatologic studies or treatment earlier or later In the follow up study an almost stiff spine was noted, inspirium expirium difference 2 cm in the right wrist thickening of volar tenosynovia Changes characteristic of chronic prostatovesiculitis ESR 5 mm/h Waaler Rose titer 0 AST 125 In a roentgenologic study ankylosis of both sacroiliacal joints was found, as well as syndesmophytes and bridges in thoraco-lumbar spine Hands wrists and feet were roentgenologically normal

Rheumatoid arthritis was noted in one case

Case 5 N V 50-year old mechanic At 36 he came to the urologic clinic because of acute prostatitis At 48 swelling and morning stiffness in fingers elbows knees and ankles In the follow up study no clinical abnormalities were noted in the joints but there were changes characteristic of chronic prostatovesiculitis In a roentgenological study of the hands little erosions were noted in one finger joint and both wrist joints ESR 3 mm/h Waaler Rose titer 0 AST 500

Among the patients 13 had had gonorrhea In one of them ankylosing spondylitis was noted

Among the patients 14 had had an inflammatory joint complaint (swelling and tenderness), one of them rheumatic fever No roentgenologic signs of rheumatoid arthritis were noted Sixteen patients had suffered from painful joints, degenerative roentgenologic changes were noted in 4 of them In one case the changes were severe Humeroscapular periarthritis with calcification was noted in one case, in one chondromatosis of the knee joint and in a third congenital hip luxation

Eighty patients complained of low back pain after excertion A roentgenologic study revealed degenerative spinal changes in 44 patients So called syndesmophytes in connection with scoliosis and disc degeneration were noted in 2 cases Reactive ' sacro iliacal joint changes (slight sclerosis without erosions) (13) in connection with scoliosis and disc degeneration were noted in 3 cases Spina biphida was found in 6, other anomalies in 6 cases

In the control cases the roentgenologic study of the spine revealed no case whatsoever of ankylosing spondylitis In 54 cases degenerative spinal disease was found Syndesmophyte-like changes in connection with disc degeneration and scoliosis were found in 3, reactive sclerosis of the sacro iliacal joints in 2 cases Anomalies were found in 8 cases

Discussion

In a group of 104 cases of non selected urologic patients a follow up study with roentgenologic study of the spine and joints revealed 4 cases of ankylosing spondylitis and one case of rheumatoid-arthritis It turned out that one case of

spondylitis and the rheumatoid arthritis case had not been diagnosed earlier. Spondylitis is a much rarer disease in Europe than rheumatoid arthritis. In Holland deBlecourt et al. (2) found ankylosing spondylitis in 0.17 per cent of the male population and in Finland Laine (8) found rheumatoid arthritis in 1.3 per cent. However, both studies were carried out by methods differing from that of the present study.

It has been suggested, in the light of a selected material of definite rheumatoid arthritis, that sacro-iliitis may be caused by rheumatoid arthritis (12) because a positive rheumatoid factor was found in 50 per cent of cases. However, in the non selected material of the same hospital the rheumatoid factor was present only in 10 per cent of the cases with bilateral sacro-iliitis (7). There was no essential difference in the test methods for the rheumatoid factor. Thus the roentgenologic finding (erosions, sclerosis, pseudo-widening, narrowing or ankylosis) of the sacro-iliacal joint argues for ankylosing spondylitis rather than rheumatoid arthritis. It is not clear whether the ankylosing spondylitis is an entity separate from rheumatoid arthritis, but the whole clinical picture is different in many ways (5). If the patient has symmetrical arthritis, it is possible, according to the criteria of ARA (1), to assert that there is a "definite rheumatoid arthritis" as described in the present cases 2, 3 and 4. Later the arthritis can completely disappear as in cases 2 and 4. Thus the ankylosing spondylitis is often hidden behind the cobweb of rheumatoid arthritis.

The etiology of ankylosing spondylitis is unknown. Urogenital infection may be of significance. The signs of prostatovesiculitis found in most cases of spondylitis may be a consequence of "auto-immune" prostatitis (4), and thus of secondary nature. However, the history revealed gonorrhea more often in the spondylitis patients than in the rheumatoid arthritis patients. The symptoms of chronic uro-genital infection were found in 11 per cent of the spondylitis cases before the age of 25. The corresponding percentage of rheumatoid arthritis cases was 13 before the age of 39 (5). Thus uro-genital infection seems to be of greater primary importance.

The occurrence of spondylitis and rheumatoid arthritis in a non-selected material of chronic salpingo-oophoritis has also been studied (6). Heredity may play a role in the etiology of ankylosing spondylitis (2, 5).

Summary

The purpose of the study was to find out what role ankylosing spondylitis and rheumatoid arthritis play in a non selected group of patients suffering from genito-urinary disease. The follow up study comprised 104 cases of 149 patients. Of them 77 were admitted because of acute or chronic prostatitis and 27 because of non specific epididymitis. The mean follow-up time was 7.5 years. Roentgenologic study of the sacro-iliacal joints, the lumbar and lower thoracic spine was done in each case and roentgenography of other joints was done in 38 cases suffering from joints symptoms. The mean age of the patients

was 46 years and the mean age at onset of the urologic complaint was 35 years

In the present study 4 cases of ankylosing spondylitis and one case of rheumatoid arthritis were found One case of spondylitis and one case of rheumatoid arthritis had not been revealed earlier In all of them a chronic prostatovesiculitis was noted

The control series consisted of 100 patients (mean age 51 years) None of them had signs of prostatovesiculitis or rheumatic symptoms No case of ankylosing spondylitis was noted in this group

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From the St Elisabeth Hospital Tilburg The Netherlands

Polymyalgia Rheumatica, Arteritis and Hepatic Damage

By

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The literature of the past few years clearly reflects the interest shown in rheumatic polymyalgia The clinical picture was first described by Meulengracht (23), and has been further defined and elaborated by Kersley (18) Forestier and Certonciny (12) Bragatuni (6 7 8) Barber (2), Gordon (13) and many others It has been described under various names such as the myalgic syndrome of the aged with systemic reaction (18) the anarthritic rheumatoid syndrome (6, 7 8) pseudo polyarthrite rhizomelique (12) Barber introduced the designation polymyalgia rheumatica which is the one most widely employed

There is little disagreement about the symptoms and manifestations of the condition which is characterized by more or less acute intense myalgia, stiffness and atrophy of the cervical and humeroscapular muscles and sometimes also of the muscles of the hip and lumbar regions The majority of authors point out that the pains are localized to the periarticular tissues of the scapular and hip regions and that there are no demonstrable signs of arthritis, others (6, 7, 8 13) have described transient swelling of the joints in a few cases The above mentioned changes are associated with general malaise weight loss anorexia, hyperhidrosis, a subfebrile temperature and depression and cause considerable disability to the patient A constant laboratory finding is a greatly increased ESR, other laboratory findings of significance are a pronounced increase in the plasma fibrinogen concentration, changes in the globulin fractions of the plasma proteins (all fractions may be increased but the increase chiefly affects the α_2 fraction) while the albumin fraction is decreased A moderate degree of normochromic anaemia is invariably found The Waaler Rose test is nearly always negative The EMG discloses no abnormalities (5)

It follows from the above that the diagnosis is in fact established by elimination In the early stages in particular, diseases to be eliminated include myelomatosis and carcinomatosis, the same applies to the well known collagen diseases such as rheumatoid arthritis, der

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matomyositis, periarteritis nodosa, lupus erythematosus disseminatus and scleroderma It is also of importance to point out that the disease is chiefly encountered in the higher age groups, and shows a female predominance The average age at onset is 58 years (Bragatuni)

The prognosis is regarded as favourable Data collected by Bragatuni show that in his patients the disease was self-limiting and the average duration of symptoms was seven years Bragatuni maintained that salicylates and physiotherapy are sufficient to obtain satisfactory improvement Other authors disagree Barker, for example, and also Gordon, reported that the above mentioned measures were inadequate in their patients, nor did they succeed with phenylbutazone and gold injections But they observed striking and almost immediate improvement following the administration of corticosteroids — an experience which many other authors have corroborated Gordon and Kersley prefer brief intermittent courses of treatment with these steroids in view of the possible side-effects, particularly in patients of advanced age others advocate continual treatment with the smallest possible maintenance dose (with a view to possible relapses)

New points of view have been put forward in the past few years They concern the aetiology of this disease and the occurrence of disturbances in liver function A brief discussion of these views seems indicated in view of the fact that the case history of a woman presented later, includes data on these two aspects

I Aetiology

The aetiology and pathogenesis of polymyalgia rheumatica are obscure, and classification of the condition consequently offers considerable difficulties Bragatuni's series of publications however maintain that the disease is an abortive form of rheumatoid arthritis (hence his suggestion that the disease be named "anarthritic rheumatoid disease) He contends that the disease is generally self limiting carries a favourable prognosis and unlike periarteritis nodosa dermatomyositus, scleroderma and lupus erythematosus disseminatus does not shorten the life span

In his opinion the signs and symptoms of the disease are strongly reminiscent of the prodromal manifestations of rheumatoid arthritis before arthritis occurs In 20 % of his patients moreover, he found a family history of acute rheumatic disease or rheumatoid arthritis, and two patients after showing symptoms for 5 years, developed a verified rheumatoid arthritis In a few cases he observed transient swelling of the joints para articular subcutaneous nodules and a weakly positive Waaler Rose test He points out that the patients are in the same age group as that of rheumatoid arthritis

Barber however, was unable to demonstrate swelling of the joints and, like Gordon he always found a negative Waaler Rose test. It must also be pointed out that an excellent prognosis is not a usual characteristic of rheumatoid arthritis and that the peak frequency of rheumatic polymyalgia comes at a later age than that of rheumatoid arthritis (22) Thus while clinical findings afford an insufficient understanding of the aetiology, biopsy material and if necessary postmortem findings may be of great value

Gordon examined a biopsy specimen from the deltoid muscle in six cases with negative results The muscle biopsy specimens examined by Kersley likewise yielded a negative result Recently Bragatuni reported in detail on 50 patients followed up from 1945 to 1961 Eleven of these died but the cause of death was not related to the disease under discussion A postmortem examination was per

formed in six of these cases The joints and the spine were examined and the majority of organs were examined for the presence of known collagen diseases and arteritis No signs of rheumatoid arthritis or other collagen diseases were found except in one patient in whom a suspicion of arteriolitis of obscure origin was found No LE cells were found during life

A biopsy was performed 17 times in 13 of the surviving patients (this includes examination of tissue and organs removed at operation) The specimens examined were obtained from muscles skin and jointcapsules liver gallbladder and prostate and (twice) from the temporal artery The examinations disclosed no distinct pathology apart from the specific changes in the organs resected because of a given disease Unfortunately no biopsy was carried out on the paraarticular nodules observed in seven patients which the author considers typical of rheumatoid arthritis

In view of the above it is obvious that the amount of available histological material is meagre and has afforded little clarification of the questions under discussion It is therefore of importance to consider another point of view which has recently received corroboration based on histological findings This view was first presented by Pauley and Huges (26 27) who contended that polymyalgia rheumatica is a clinical form of giant-cell arteritis Giant-cell arteritis is a systemic disease chiefly localized in the medium size arteries and usually in those of the carotid region in particular in the temporal artery Hence the original name temporal arteritis (Horton) The condition is not confined to the arteries of the carotid region but can also be encountered in such arteries as the subclavian vertebral coronary mesenteric and and renal arteries The characteristic changes found in these arteries are granulomatous inflammatory lesions with giant cells in the media, fibrinoid degeneration of the media fibrosis of the intima and microcellular infiltrates in the adventitia (9 10 16 21 28)

Apart from general symptoms such as fever malaise anorexia and signs such as a relatively marked increase in the ESR the clinical picture is chiefly determined by the localization of the arteries affected, the ocular anomalies are the features most widely known Patients with this disease may show a syndrome of myalgia atrophy and stiffness which is indistinguishable from that of rheumatic polymyalgia Moreover signs and symptoms of temporal arteritis in patients with giant-cell arteritis can occur late, be transient or even absent On the other hand the temporal artery can show the histopathological changes in the absence of signs or symptoms localized in the temporal region (25) In this condition muscle biopsies nearly always yield a negative result Also giant-cell arteritis and rheumatic polymyalgia show a preference for the same age groups and patients showing the myosyndrome of giant cell arteritis show an excellent response to corticosteroids (Ross-Russell)

Thus while there are clinical arguments in favour of the theory that rheumatic polymyalgia may be a clinical form of giant-cell arteritis it remains difficult to establish this without demonstrating the common histopathological substratum We can well understand Bragatuni (8) who could not demonstrate such a substratum writing Biopsy or postmortem proof of giant-cell arteritis is the only valid reason for calling a disorder by that name or its synonyms

Recently however Alestig and Barr (1)and Hamrin et al (14) obtained such proof The former obtained a biopsy specimen from the temporal artery in five females and four males who showed the typical signs and symptoms of rheumatic polymyalgia and averaged 70 years of age None of these patients had palpable abnormalities or pain in the temporal region Seven yielded biopsy specimens which showed the characteristic features of giant-cell arteritis Hamrin et al performed 36 arterial biopsies in 21 out of 23 patients with polymyalgia rheumatica seen in the past two years A segment of the temporal artery was resected in 21 cases an additional specimen was obtained from the scapular circumflex artery in 6 patients from the perforating branch of the femoral artery in 5 from the superior gluteal artery in 1 and from the

occipital artery also in 1 patient The 23 patients included 9 with signs suggestive of temporal arteritis The laboratory findings show that in 15 patients the ESR exceeded 100 mm after one hour, while in 8 patients it was below 50 mm The plasma fibrinogen concentration was greatly increased in all the patients A study of the plasma proteins disclosed a slight increase in γ globulin in 7 patients an increase in α globulin in all cases and frequently a decrease in the albumin fraction The majority of patients had a normochromic anaemia Unmistakable eosinophilia was found in a considerable number of the patients A study of LF cells was made in 10 patients with negative results The Waaler Rose test was negative in all patients except one X ray examination of the shoulders and hipjoints disclosed periarticular calcifications in the soft parts in 4 of the 21 patients X rays of the spine were normal

Out of 12 patients without signs or symptoms in the temporal region there were six whose temporal artery biopsy specimen showed the typical features of giant-cell arteritis and one whose specimen indicated non specific arteritis Of the 9 patients whose signs and symptoms indicated temporal arteritis 6 yielded temporal artery biopsy specimens with the features of giant-cell arteritis the remaining 3 biopsy specimens showed signs of non specific arteritis Specimens from the superior gluteal artery and the perforating branch of the femoral artery in one case showed the features of non-specific arteritis the same was seen in a specimen from the occipital artery The scapular circumflex artery specimen showed no changes

These observations demonstrate firstly that there is clinical relationship between the syndromes of rheumatic polymyalgia and giant-cell arteritis They also warrant the conclusion that in many cases the two diseases have a common histopathological substratum Thus the view that polymyalgia rheumatica is a clinical form of giant-cell arteritis is given important corroboration But this does not solve the problem of the aetiology because the cause of giant-cell arteritis is also unknown The histological features (frequent reports of fibrinoid degeneration), the clinical picture the increased ESR changes in plasma proteins and favourable response to corticosteroids lend support to the hypothesis that giant-cell arteritis might be one of the collagen diseases But such a hypothesis demands further investigation on the basis of clinical genetic and epidemiological data and findings obtained by immunological techniques the data thus obtained will be decisive In this context it may be pointed out that arteritis forms part of the pathology of the known collagen diseases and may be an expression of auto-immunization (30)

V Disturbances in liver function

In 1963 Klijn and Boomgaard (19) described three patients suffering from polymyalgia rheumatica in whom one or several liver functions were disturbed These patients showed a pathologically increased bromsulphalein retention two in addition showed increased serum alkaline phosphatase values The authors maintained that these liver function disturbances are part of the disease However one of these patients whose liver puncture was normal was treated for cholelithiasis by cholecystectomy Cholesterol crystals were found in bile from the second patient The third patient was febrile during the investigation the isoenzyme pattern of the alkaline phosphatase was not determined and the cause of the increase consequently remained obscure On the basis of these facts the authors conclusion although not untenable must be regarded as unproven

In this connection it would seem useful to present the case history of a woman in whom evidence was obtained of disturbances in liver function and (electron microscopically) of organic hepatic changes and who yielded a temporal artery biopsy specimen in which signs of arteritis were evident

Case report

A 50-year-old woman was hospitalized with a two-month history of gradually increasing pain and stiffness of the shoulders neck and

thighs The joints had not been red or swollen The patient had lost weight she had developed anorexia and hyperhidrosis and finally had become unable to look after herself She had no complaints about the temporal region or about her vision

Physical examination showed a woman in reasonable nutritional condition passively confined to bed and giving the impression of being ill The temperature fluctuated between 38 C and 39° C The muscles of the neck humeroscapular region and thighs were stiff and very painful this greatly reduced mobility The joints per se were not swollen or red Further examination showed no abnormalities of the heart or lungs the lymph nodes were not swollen and the liver and spleen were not palpable There was no jaundice The temporal artery was not tender and showed bilateral pulsations Neurological findings were normal The EMG (electromyographic examination) of the trapezius and deltoid muscles was likewise normal The ocular fundi showed normal features

X ray examination

X rays of the chest shoulders hands hip joints and knees disclosed nothing abnormal An intravenous cholangiogram showed that the bile ducts and gallbladder were normal

Laboratory investigations

Urine albumin — reduction — urobilinogen + + sediment normal ESR 108 mm in one hour Hb concentration 11 1 g/100 ml Red cell count 3 9 million colour index 0 89 mean diameter 6 8 μ Leucocyte count 10 800 (eosinophils — staff cells 1 5 %, segmented cells 58 5 %, lymphocytes 36 %, monocytes 4 %) The Wassermann Kahn and VDRL tests were negative AST < 50 U Rose test O L agglut O LE cells were not found in blood repeatedly examined for this purpose Tests for antinuclear factors with the aid of the fluorescence technique were negative Protein pattern (paper electrophoresis) total protein 65 6 g/l albumin 49 2 % α_1-globulin 7 4 % α_2 globulin 14 1 β globulin 10 4 % γ globulin 18 9 % With agar electrophoresis the α_1 α_2 and β_2 globulin fractions were clearly increased while the β_1 and γ globulin fractions showed a slight increase Fibrinogen 7 05 g/l Uric acid 3 2 mg/100 ml Cholesterol 2 18 g/l esters 72 9 % calcium 9 8 mg/100 ml Serum iron 88 μg/100 ml Total iron binding capacity 312 μg/100 ml Urea clearance (standard) 80 % Duodenal intubation yielded B-bile with a normal sediment The glucose tolerance curve was normal

Liver functions

Hymans van den Bergh total 0 29 mg/100 ml direct reaction negative Thymol turbidity test 0 2 U HgCl titration 1 12 ml Alkaline phosphatase 35 5 U (King Armstrong) Determination of the isoenzyme pattern (Haye and de Jong (15)) showed that bands II and III (the liver factors) were increased SGOT activity 45 U SGPT activity 107 U LDH 150 U with a normal isoenzyme pattern Oral galactose test 2 g excretion after 2 hours (2 8 %) A retention of 16 5 % was found 45 min after a bromsulphalein tolerance test

Pathological anatomical findings (P M Pijpers)

Biopsy from the temporal artery A thickened atheromatous intima was found in the various sections In the media and more pronounced in the adventitia a lymphocytic infiltration was found with intensive proliferation of histiocytic epithelioid cells with a pale abundant cytoplasm No polynuclear giant cells were seen (fig 1)

Biopsy from the trapezius muscle The striated muscle fibres were arranged in normal fasciculi with the striation intact everywhere The connective tissue showed normal capillaries arterioles and venules without discernible changes No degenerative changes were observed

Bone marrow puncture A slightly hyperplastic bone marrow was found The lymphocyte count was increased Numerous reticulum cells contained brown granular pigment which stained positively with Perl s staining their number was considerably above normal In stained smears erythropoiesis showed a shift to the right and leucopoiesis was



Fig 1 Arteria temporalis biopsy Hematoxylin eosin Lymphocytic infiltration in adventitia and media No multinuclear giantcells × 100

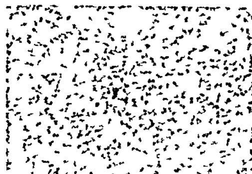


Fig 3 Liverbiopsy Hematoxylineosin Pericentral area Normal liverstructure × 250

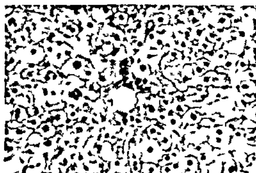


Fig 2 Liverbiopsy Hematoxylineosin. Periportal area Normal liverstructure × 100

pronounced with toxic granulation in the leucocytes the number of megakaryocytes platelets and plasma cells was normal

Liver biopsy

Light microscopical examination showed the liver to have a normal trabecular structure The nuclear structure of the cells was normal throughout and the cytoplasm was clear and vacuolated The reticulin network was normal. There were no inflammatory changes no vascular disease and no proliferation of the connective tissue The amount of pericentral lipofuscin pigment did not exceed the normal With PAS staining the liver cells were found to be full of glycogen Perl s staining disclosed a trace of intracellular iron pigment The liver cells contained pericentral microglobular fat which was not "birefringent in polarized light (figs 2 and 3)

Electron microscopical examination of liver biopsy matrial (U Van Haelst and A M Stadhouders Roman Catholic University, Nijmegen)

For electron microscopical examination biopsy material was fixed in osmium tetroxide (2 % OsO_4 veronal acetate buffer pH 7 4) and embedded in Epon 812

The sections were given contrast with lead hydroxide and examined in a Philips EM200 *microscope*

Only a few of the cytological details considered abnormal in the parenchymal cells, will be described here The most prominent abnormality concerned the mitochondria

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Fig 4 a and b Giant mitochondrium with filamentous most probably crystalline inclusion bodies in longitudinal section (4 a) In cross-section (4 b) the filaments appear as dark granules. Note the strictly regular arrangement of filaments in their preferential location along the walls of the mitochondrium The close proximity between filaments and cristae can be seen. In the cytoplasm electron dense glycogen particles are scattered between dilated endoplasmic reticulum vesicles In the lower left some clusters of ribosomes are present Villous-like projections in the space of Disse are seen at the bottom left 4 a × 40 000 4 b × 37 000

Fig 5 Crystalline like inclusion body more centrally located in the matrix of a mitochondrium Some remnants of organized rough endoplasmic reticulum are present in the cytoplasm × 47 000

4b 0.5µ

4a 0.5µ

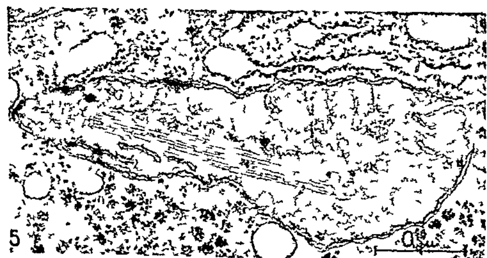




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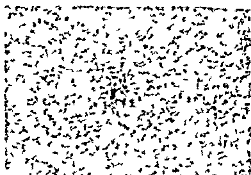


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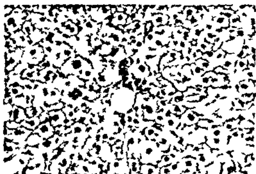


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Fig 4 a and b Giant mitochondrium with filamentous most probably crystalline inclusion bodies in longitudinal section (4 a) In cross-section (4 b) the filaments appear as dark granules Note the strictly regular arrangement of filaments in their preferential location along the walls of the mitochondrium The close proximity between filaments and cristae can be seen. In the cytoplasm electron dense glycogen particles are scattered between dilated endoplasmic reticulum vesicles In the lower left some clusters of ribosomes are present Villous like projections in the space of Disse are seen at the bottom left 4 a × 40 000 4 b × 37 000

Fig 5 Crystalline like inclusion body more centrally located in the matrix of a mitochondrium Some remnants of organized rough endoplasmic reticulum are present in the cytoplasm × 47 000

many of which contained paracrystalline inclusions (fig 4 a and b) Dependent on the manner in which these "crystalloid inclusion bodies were cut the matrix of the mitochondria showed either a parallel arrangement of alternating light and dark filaments (fig 4 a) or moderately electronscattering particles arranged in a grid structure (fig 5) The dark filaments were about 60 Å thick and sometimes 3—4 μ long The width of the clear bands in between was 60—150 Å Due to the presence of these crystalline structures the mitochondria were of abnormal size (3—5 μ) and often showed angular contours A predilection for the crystals to be situated along the external membrane was evident The number of cristae in these mitochondria seemed to be decreased Such cristae as remained were positioned on or associated with the crystal in a distinctive manner

A second prominent abnormality concerned the ergastoplasm which normally consists of an arrangement of parallel lamellae carrying RNA particles Although such particle-carrying membranes did occur scattered through the cytoplasm there was no evidence of any organized arrangement The number of free ribosomes moreover was relatively small The "smooth variety of endoplasmic reticulum on the other hand was present in normal amounts but often dilated The numerous glycogen particles showed the normal rosette structure they were not arranged in so called glycogen fields as is normally the case but were found scattered diffusely throughout the cytoplasm (fig 6) Fat vacuoles were not numerous The Golgi system of the cells showed no details worthy of special note The fibres of the Golgi apparatus lay in polar positions in the liver cells (i e between nucleus and bile capillaries) The bile capillaries were not dilated and had nothing in them discernible by means of the electronmicroscope The microvilli were normal in number and morphology The bile capillaries were sealed off from the normally wide intercellular spaces by desmosomes. Lipofuscin granules were found in many cells but the ultrastructure of these granules warrants no special comment (fig 7)

The lumen of the sinusoids sometimes contained cellular debris originating from liver cells (e g glycogen rosettes mitochondria and endoplasmic reticulum vesicles) Parenchymal cells with signs of necrosis were not found in the material despite careful search Nucleus and nucleolus had a normal appearance Although collagen fibres were frequently encountered in Disse s spaces the electronmicroscopical findings did not enable any conclusions to be made regarding these fibres

Treatment and course

Treatment consisted in the administration of 5 mg prednisone three times daily The symptoms showed immediate improvement the temperature became normal in two days After a few days the patient could move freely only mild stiffness in the shoulders persisting After one month of prednisone treatment the following laboratory findings were obtained.

ESR 52 mm Hb concentration 13 3 g 100 ml Red cell count 4 42 million Protein pattern total protein 76 6 g l Paper electrophoresis albumin 41 1 % α_1-globulin 7 6 % α_2 globulin 12 6 % β-globulin 11 4 % γ globulin 14 3 % Hymans van den Bergh total 0.23 mg 100 ml direct reaction negative Thymol turbidity test 0 5 U HgCl titration 1 48 ml Alkaline phosphatase was normal (11 6 King Armstrong U) as were serum transaminase activities (SGOT

Fig 6 A bile-canaliculus (b c) with apparently normal structure is shown between two hepatocytes. The Golgi system (G) is partly disorganized. Abundant glycogen particles are present diffusely scattered throughout the cytoplasm Two lysosomes (L are shown arrow × 22 000

Fig 7 In this micrograph part of a lipofuscin containing hepatocyte is shown Note the overall ultrastructural disorganization × 11 000

10 U, SGPT 26 U) No bromsulphalein could be detected in the serum 45 minutes after its administration Disturbances in liver function, therefore, were no longer demonstrable After 13 months of prednisone medication the ESR dropped to 11 mm

Discussion

The above case history shows that this case met all the requirements for a diagnosis of polymyalgia rheumatica as regards the signs and symptoms and biochemical changes In this patient too, the muscle biopsy and EMG (electromyographic examination) had been normal The response to prednisone was prompt and favourable Two aspects, however, merit further discussion, namely the changes in the temporal artery and the disturbances in liver function Although there were no temporal signs or symptoms, the biopsy specimen from the temporal artery showed arteritis of a non specific nature This finding is in agreement with the previously mentioned observations of Hamrin et al (14)

The disturbances in liver function must next be considered The clinical data show that bromsulphalein retention was pathologically increased and that the alkaline phosphatase was increased and displayed an isoenzyme pattern conclusive of a hepatic pathogenesis in addition the serum transaminase activity was increased, particularly the SGPT activity and the oral galactose test was disturbed

Light microscopical examination of the liver tissue failed to demonstrate an organic substratum The liver puncture was normal Electron-microscopical examination, however, showed organic changes in the liver, particularly in the ultrastructure of the liver cells These changes chiefly involved the mitochondria and the ergastoplasm but also included an abnormal glycogen distribution and abundant lipofuscin So far as we can ascertain, this is the first case of polymyalgia rheumatica in which disturbed liver functions and organic liver changes have been demonstrated In this context, however, we must establish whether these organic hepatic changes are a consequence of the polymyalgia rheumatica or of an incidental hepatopathy of different aetiology

The literature indicates that the changes described, particularly the mitochondrial changes, are not specific but have been reported in a number of different hepatopathies These mitochondrial changes have been described in chronic alcoholism (29), extra hepatic biliary obstruction (11), carcinoma of the common duct with jaundice as a result of virus hepatitis (17) and diabetes mellitus (20) Mugnaini (24) maintains that this anomaly is a nonspecific reaction of the chondriosome to a cell injury which affects the normal enzyme activity of the mitochondria In our patient, the above mentioned diseases could be excluded on the basis of the clinical features and course, and the normal appearance of the liver tissue at microscopy This also applies to infectious hepatitis, among other reasons because both the light microscopical and the electron microscopical examination disclosed a normal nuclear and nucleolar appearance — in contrast to the changes described in infectious hepatitis by Bearcroft and Peachy (4) and Bearcroft (3)

On the basis of these facts we may conclude that the disturbances in liver function and the hepatic changes in this patient were a result of the polymyalgia rheumatica, but cannot be described as specific for this disease

Summary

A discussion of the clinical picture and biochemical features of polymyalgia rheumatica is followed by a review of recent views on the aetiology and the occurrence of disturbances in liver function The case history of a woman is presented In this case, the existence of disturbances in liver function and organic liver changes was demonstrated for the first time (the latter by electron-microscopical examination) This patient also suffered from a nonspecific arteritis of the temporal artery

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Hereditary Renal Disease Associated with Deafness — Alport's Syndrome

By

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The older literature mentions a number of families in which several members suffered from renal disease e g Dickinson (3) and Pel (13) Alport (1) however after studying four generations of one and the same family was the first to call attention to a particular hereditary nephropathy, often accompanied by deafness

Clinical manifestations

Alport s syndrome is distinguished by the clinical signs and symptoms the characteristic course and the mode of inheritance Usually the patients are asymptomatic for many years and they are able to pursue their daily activities without any difficulties until a relatively short time before the possible fatal issue Haematuria is the only abnormality found in the beginning during the night s rest this is less marked (11) and it often becomes more manifest during (viral) infections (25) This haematuria has been found as early as in the first months of life (1 11) but in some periods there are only few erythrocytes present in the urine The haematuria is usually attended by a greater or lesser degree of albuminuria and sometimes by cylindruria, granular or hyaline cylinders Some investigators (14) emphasize the presence of urinary tract infections but in the majority of cases described (1 6, 12 17 18 21 25 and in our patients) there were none Initially there are no other abnormalities in particular no disturbances of renal function the intravenous pyelograms sedimentation rate and blood pressure are normal On the other hand the serum α_2-globulin is often elevated (2 16 21 22 25) In many cases perception deafness develops at the age of about 7—11 (4 10 20 25) this may ultimately lead to serious deafness In these families the deafness may occur either with or without renal abnormalities

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Fg 2 Survey of the cortex few glomeruli atrophic and dila ed tubuli ncrease of connec tive issue H—E, ×120



Fg 3 T o glomeruli one w h foetal structure, the other w th h alinizat on, adhesions to Bowman s capsule H—E, ×300

because haematuria was found He had no complaints and had never been ill The urine contained prote n and quite a number of erythrocytes Blood pressure urea clear ance concentrat on power the phenolsulph thalein excretion the ntravenous p elograms and the sedimentation rate were normal At the age of 21 the sed mentation rate was somewhat ncreased (13 mm after one hour) A year later the blood urea was somewhat too h gh (46 mg %) the blood pressure was st ll normal (135 85 mm Hg) the serum cholesterol was 272 n g % At the age of 24 the blood pressure was still somewhat too high (155/105 mm Hg) the serum urea was 60 mg % creat n ne 2 1 mg % choles terol 295 mg % total prote n 6 05 g % of wh ch album n comprised 66 % α_1 globulin 3 % α_2 globulin 6 % β globul n 12 5 % γ globul n 12 5 % haemoglob n content 15 g % sedimentat on rate after one hour 15 mm Urine prote n ++ Sed ment some erythrocytes and some h aline cylinders The pat ent had no complaints at all his hearing was good and he d d h s work regularly The aud ogram was normal He had no oedema Half a year later the blood pressure was 175 105 mm Hg serum creat n ne 3 1 mg % sed mentation rate 25 mm af er one hour *The fundus ocul was normal* the ret nal vessels were of a normal s ze Dur ng his last year the pat ent had suffered much from headache and mild nose bleeds The blood pressure rose to 210/120 mm Hg the serum urea rose rap dly (87—164—387 mg %) as did the creatinine content (4 8—13—3? mg %) and a serious anaem a developed Not until then did the ECG show ind cations of left hypertrophy and after e ght months the p cture of left stra n The urine contained no prol ne or hydroxy prol ne the serum conta ned 4 mg % pro-l ne Vis on deteriorated due to cystic degen erat on of the macula and in both eyes a retinal haemorrhage was vis ble The pat ent de eloped manifestations of left and right congest e heart failure and died at the age of 26 At autopsy (S 4471) of this thin man a cons derably enlarged heart was found weighing 630 g The left ventricle had a thick wall Due to the failure of the left heart the lungs were congested The l ver was much enlarged we gh ng 2300 g the spleen we ghed 130 g Both organs showed s gns of congestion which must be connected with the right congest ve heart failure The enlarged heart must have been the result of hypertens on caused by the renal affect ons

The ad nals were large and we ghed al together 28 g The hypertrophy is probably secondary n nature related to the h per tens on and disturbances of the renal funct on

The mesentery contained an open ng the s ze of a f st part of the small intestine had sl pped through it

The k dneys were small we ghing alto-gether 130 g The capsule was read l detachable f om the finel granulated sur

face The cortex was thin (2—3 mm) The pyela were slightly widened and presented three calyces Ureters and renal arteries did not present any noteworthy features The urinary bladder was enlarged and contained clear urine, the mucosa was pale

On microscopical examination it was noted that there were only few glomeruli These were rather big and the majority of them still had foetal characteristics (figs 2 and 3) They contained hyaline spots Here and there adhesions to Bowman s capsule, which was lined with swollen cells, were visible The tubuli were atrophic or hypertrophic (fig 2) The latter were situated in little groups and had caused the fine granulation of the surface The hypertrophic tubuli were dilated and lined with flattened or swollen epithelium Several tubuli contained a protein cylinder, an occasional one contained calcium The interstitium showed a marked increase of connective tissue and quite an amount of lymphocytes infiltrate Foam cells were not found The arterial walls were thickened The medulla varying in width, contained a decreased number of dilated, slightly irregular collecting tubules in which were protein cylinders

The two sons of patient A s sister suffered from haematuria The eldest patient B was symptom free at the age of 16 Urine protein + sediment some erythrocytes per visual field Blood pressure 135/85 mm Hg Sedimentation rate 4 mm in 1 hour serum urea 30 mg %, creatinine 0 72 mg % normal *creatinine clearance* cholesterol content 232 mg % Eighteen months later the condition was practically the same (sedimentation rate 3 mm in 1 hour serum creatinine 0 70 mg %) but the blood pressure was somewhat high (152/92) He had no complaints A bilateral perception deafness was *found in this patient* (16)

The youngest brother patient C had been extensively examined by Dr Peters (16) at the age of three He had haematuria but no albuminuria normal urea and creatinine clearances normal intravenous pyelogram sedimentation rate 10 mm in 1 hour The α_2 globulin content was 11 9 % serum cholesterol 196 *mg* % *The mother of these boys (case D), 41 years old, had haematuria, albuminuria and a bilateral perception deafness, but no complaints*

The mother of patients A and D (patient E) had albuminuria and haematuria at the age of 69, but no complaints Two years later she developed gouty arthritis of the left metatarsophalangeal joint without further complaints At this time she had hypertension (215/120) and her renal function was disturbed (serum creatinine 1 88 mg %, urea 57 5 mg %, urea clearance 33 %) the uric acid content of the serum was 7 1 mg % She had sustained ten pregnancies without any difficulties

However there was some convergence, being exceptions in both sexes, to this characteristic difference in the course of the renal affections In the first place, in a few families the course in the males is not so unfavourable (15), as also demonstrated by the family described by Ohlsson (12) The four male members of this family had albuminuria and/or haematuria, just as had 2 of the 3 females Three of the men, 37, 32 and 21 years old, had perception deafness the blood pressure was normal and in only one of them was the renal concentration power disturbed In all three the urea and creatinine levels of the serum were normal

On the other hand, some families have been described in which also the women presented an early serious course (1, 2, 22, 23) One of us has been in the position to perform autopsy on two women from one family, both had died from this nephropathy at the age of 32

In addition to the renal affections and the disturbed hearing ocular diseases have sometimes been observed in these patients viz , congenital nystagmus (2),

cataract (4, 6, 20), spherophakia (20), and serious forms of myopia of 20—22 diopters (12)

Although in typical cases of this disease, on many occasions no abnormal amino aciduria has been observed (6, 8 14, 16), in some families the patients (12, 19, 22), and sometimes also relatives without disturbed renal function (19, 22), showed abnormal amino aciduria Wallace (22) speaks of general amino-aciduria' Schafer (19) found hyperprolinaemia and prolinuria and hydroprolinuria In two of Ohlsson s (12) patients an increased excretion of alanine, glutamic acid, histidine and threonine was found Schafer s (19) patient a five year old boy, had in addition to perception deafness and renal abnormalities serious cerebral disturbances and he died at the age of five following anuria

Heredity

When studying the heredity, a distinction should be made between the renal disease and the perception deafness Although the two quite often occur in combination this is not a general rule On the one hand there may be deafness without renal disease and on the other hand there were patients, including serious cases, who died from the renal insufficiency (23) but did not suffer from deafness No prognostic significance can therefore be attributed to the deafness Deafne s occurs more frequently in males than in females Perkoff (15) found a 20 1 ratio Reyersbach (18) one of 11 3 In patient A s family in the fourth generation (fig 1) four of the ten siblings had renal abnormalities and two hearing disturbances, of the latter two, one suffered from both

Our findings are consistent with the generally accepted concept that the renal disease is conditioned by a dominant gene in a heterozygous carrier Furthermore, here also it appears that the renopathy is, as a rule transmitted by the mother, and the gene is therefore thought to be bound to the X chromosome In spite of this, occasionally a father to son transmission has been observed (15) Hence that Perkoff thinks that there may be a partially sex-bound dominant gene and the possibility of a crossing-over to the Y chromosome It should be taken into account that the disease seems to be so rarely transmitted by men because most of the male sufferers die before they have had offspring Moreover, it has been observed that the daughters of a male patient did not always present renal abnormalities These were still young however (4—16 years) (15), and it cannot yet be ruled out that they may prove to be carriers

If the gene is localized on the X-chromosome the difference between sexes in the course of the disease might be explained by the presence in the female, of a modifying gene localized on the other X-chromosome (7)

If so the rare instances of a serious course in females might concern homozygotes Indications of this are 1 in the family described by Alport (1) in which a 24 year-old women died from the renal disease six of the seven siblings showed renal abnormalities and four hearing disturbances three of them being very deaf 2 Whalen s (23) patient

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In addition to the renal affections and the disturbed hearing ocular diseases have sometimes been observed in these patients, viz , congenital nystagmus (2),



Fig 4 Proximal tubuli with degenerative characteristics H—E ×45



Fig 5 Collecting tubule with abnormal, thin connecting pieces H—E ×45

extensive staphylococcal infections of the kidney, who did not present Alport s syndrome It was noted, though, that in these cases often several members of the family suffered from glomerulonephritis

In addition to the lymphocytic infiltrations, in some cases polynuclear leucocytic infiltrations, occasionally even with abscess formation, have been described at the transition from medulla to cortex which suggested pyelonephritis, but sometimes one had the impression that this condition was superimposed (6 23)

This anatomical picture repeatedly in evidence is not specific for the well known renal diseases like chronic glomerulonephritis pyelonephritis and arteriolosclerotic contracted kidneys and it explains why most authors consider it difficult to classify this picture

One of us carried out microdissection of the kidneys in our patient A This technique was attended by many difficulties due to the great quantity of interstitial connective tissue It was impossible to extirpate an entire nephron, since the glomeruli usually broke off Nor was it always possible to follow the distal part of the nephron in a proximal direction

After many attempts however with concentrated HCl such a degree of maceration was obtained that it became possible to acquire an impression of the structure of the nephron

There were only few glomeruli Their size was variable, and occasional ones were black, attached to thin, black proximal tubuli wound into a small ball The length and especially the thickness of the proximal tubuli were rather variable (fig 4) some were broad with a winding course The terminal part was rather convoluted Thin tubuli were also found In general their length was too small It was hard to discern Henle s loops This part of the nephron broke off very easily It can be said however that Henle s loops were convoluted

The most important abnormalities were found at the distal part of the nephron The collecting tubuli were in general thickened and of a rigid consistency many of them contained cylinders These were also found in the distal



Fig 6 Collecting tubule with a few diverticles H—E ×60



Fig 7 Collecting tubule with diverticles and a thin connecting piece H—E ×45

tubuli The collecting tubuli had few ramifications (fig 5) usually situated at the end with thin connecting pieces (fig 7) There were only a few central ramifications Distally small diverticles were seen (figs 6 and 7) The distal tubuli were thickened rigid with a yellow tint and an S shaped structure There were few windings Here and there the thickened parts alternated with thin ones (fig 8)



Fig 8 Collecting tubule with two distal tubuli with constrictions (↓) and dilatations H—E ×45

Pathogenesis

In view of the family history it is established that we are dealing here with a congenital disease Time and again attention is drawn in the literature to the strikingly small number of glomeruli present a fact confirmed by us

In three cases in which microdissection of the kidneys was applied by us important abnormalities were found especially of the distal part of the nephron The collecting tubuli have had too few central and peripheral ramifications which moreover had an abnormal course Furthermore there are diverticle like protrusions The proximal tubuli are too small The variable structure is caused by the differences in the degree of atrophy and hypertrophy The black tubuli are probably degenerating nephrons The increase of connective tissue with lymphocytic infiltration can be regarded as a reaction to the degeneration of renal tissue

All these data render it probable that there exists a developmental *disturbance* of the kidneys This concerns the development of the ureteral bud as well as that of the metanephrogenic tissue Probably the number of divisions of the ureteral bud had decreased with a poor induction of the metanephrogenic tissue

The lay-out of the kidney, as a whole, is defective as is shown by the decreased number of ramifications and the diverticle formation of the collecting tubules, the short proximal tubules and the abnormalities of the glomeruli Due to the decreased number of nephrons the renal function may become insufficient It is understandable that the renal tissue present tries to adapt itself by hypertrophy of the tubuli which, in the long run, may lead to excessive organ fatigue and to degeneration of the nephrons This is indicated by the degenerated black discolored proximal tubuli and glomeruli

This concept is consistent with the observations that in renal biopsies, carried out at early age, no or only mild abnormalities were found no increase of connective tissue and no lymphocytic infiltrations (11, 14, 18) Also the clinical course becomes understandable seen from this angle

During the first 10—20 years of life it is usually impossible to demonstrate disturbances either of glomerular filtration (urea and creatinine clearance) or of the tubular function (concentration power, phenolsulphthalein excretion, intravenous pyelograms) Apparently the limitation of the number of nephrons in the male patients is usually stronger than in the females for if the number of nephrons were not so strongly limited the patient might reach a normal duration of life and might even go through a great number of pregnancies without serious consequences The haematuria and albuminuria, which are usually found at an early age and which persist more or less throughout life might be connected with abnormal glomerular structure

In earlier days it had already been suggested (5, 9) that dysplastic kidneys would be especially sensitive to infections This might explain that not rarely inflammatory processes have been found in sufferers in a further advanced stage of the disease, this gave the impression of pyelonephritis which, as Goldbloom (6) remarked may mask the hypoplasia or dysplasia of the kidneys

Summary

Based on the case history of a patient who has been observed for ten years and on whom autopsy was carried out, a survey is given of the signs and symptoms and the course of Alport s syndrome The results of the heredity study in this patient s family are consistent with the idea that the heredity of the renal abnormalities is conditioned by a dominant gene that is partially sex bound Usually the course in men is much more unfavourable than in women even though there are a few female patients with a serious course There are indications that these patients were homozygotes

The results of the pathological examination by means of microdissection techniques render it probable that in Alport s syndrome there exists a congenital developmental disturbance of the kidneys This involves the whole nephron but becomes most manifest in the distal part Thus the kidneys sooner or later become insufficient This tallies with the negative results of biopsies made at early age and with the characteristic course of the syndrome

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Angina Pectoris and Exposure to Cold

By

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It is a fairly common experience that some patients suffering from angina pectoris are particularly troubled by pain when exposed to cold When questioned on the matter, these patients characteristically relate that their pains may occur just after leaving home on cold days during the winter season The temperature outside is most often below 0° C, but not necessarily many degrees Often the low temperature is combined with some wind The pains may start before the patients have been physically active, or at least on less activity than is normally sufficient to produce pains After staying in the cold for some minutes, they can usually continue their activity

There is no explanation of the phenomenon in the literature No doubt the pains are true angina pectoris and accordingly the pathogenesis must be a relatively insufficient oxygen supply to the heart muscle The problem is how exposure to old can be the etiological basis for this insufficiency

Etiological possibilities that should be considered are

1 Increased oxygen consumption in the myocardium

2 Decreased blood flow through the coronary arteries caused by a possible vasoconstriction

3 Reduced oxygen content of the arterial blood caused by a reduced uptake of oxygen in the lungs

An attempt has been made to elucidate these possibilities A number of patients with known angina pectoris have inhaled cold air (— 14° C) under the following conditions

1 Bed rest for 20 minutes

2 Room temperature + 20° C

3 At least 2 hours since the last meal

Method

1 Pulse and respiration rate blood pressure ECG and oxygen saturation of arterial blood were registered

2 With a clip on the nose the patients inhaled fresh air at — 14 C by mouth (see fig 1)

3 After 3 5 and 10 minutes inhalation pulse and respiration rate blood pressure ECG and oxygen saturation of arterial blood were registered

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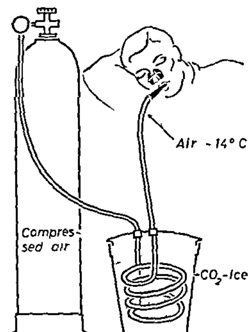


Fig 1 Compressed air is cooled by passage through a system of isolated plastic tubes and a copper spiral embedded in CO_2 ice

The results of these experiments are shown in table I Of the 15 patients examined, none showed any changes in pulse rate or blood pressure or any reduced arterial oxygen saturation In one patient only, the ECG showed hypoxia changes Among the 15 patients 7 (8) had a positive angina pectoris — cold history and 6 of these had pains during the experiment

Discussion

From the data given, it seems unlikely, that cold exposure causes increased heart activity and thereby and increased need for oxygen The arterial oxygen saturation was not found to be significantly changed in any of the patients during the experiment Should cold air be able to produce, by a reflex mechanism, a constriction of the coronary arterise, this might be a possible explanation of the phenomenon Constriction of the coronary arteries is caused by parasympathetic nerve fibres in the vagus nerve Allen (1) has recorded respiratory inhibition, blood pressure elevation, bradycardia and increased pulse amplitude in response to vaporous stimulation in rabbits and cats Frankenhaeuser and Lundervold (3) have described a reflex inhibition, in a rabbit, of spontaneous muscular action potentials from leg muscles and accessory respiratory muscles when ether was placed in front of the animals nose Andersen (2) found the afferent part of this reflex to be mainly the trigeminal nerve

It is beyond the scope of this paper to discuss probable linkages between the vagus nerve and afferent nerve fibers from afferent nerves in the nose, mouth and face Anatomically, impulses from these regions can probably follow nerve fibers belonging to the 5th and 9th cranial nerves The anatomical relationship between the nuclei of these nerves and the vagus nerve makes a connection possible

Summary

Why some patients with angina pectoris are more troubled with pains when exposed to cold air is not known Under standardized conditions, 15 patients inhaled fresh air at — 14° C No rise in blood pressure, pulse or respiration rate, and no decrease of the arterial oxygen saturation could be registered Six patients suffered pains during the ex

TABLE I The effect of cold air inhalation on patients with angina pectoris (A.p)

Patient	Diagnosis	"Cold pains in history	Pains during experiment	Changes in Pulse frequency	Changes in Blood pressure	Changes in Arterial O_2 saturation[1]	Changes in Hypoxia changes in ECG
1	A p	—	—	— 4	130/70—125/70	— 2 2 %	—
2	A.p and xanthomatosis	—	+	— 0	140/50—140/90	+ 2 5 %	—
3	A p and infarction	+	+	+ 4	135/85—130/80	0	+
4	A.p	—	+	+ 2	160/100—155/90	0	—
5	A p	+	—	+ 2	150/90—155/95	0	—
6	A p	+	+	— 2	170/100—160/95	— 1 2 %	—
7	A.p	—	—	— 0	135/95—140/100	0	—
8	A p	+[2]	+	— 3	130/80—135/90	0	—
9	A.p	+	—	— 3	175/100—165/80	— 2 %	—
10	A p	+	—	+ 2	145/95—150/100	0	—
11	A p	—	—	+ 3	125/85—125/95	0	—
12	A p	—	—	— 0	165/90—165/95	0	—
13	A p	+	+	— 2	155/100—1 0/100	+ 1.2 %	—
14	A p	+	—	+ 2	165/95—170/100	0	—
15	A p	—	—	+ 2	145/100—150/95	0	—

[1] The estimated values for the arterial O_2 saturation were all between 90 and 95 %

periment but only one showed ECG changes It is suggested that a reflex mechanism via the 5th and the 9th cranial nerves to the vagus nerve, thus causing a constriction of the coronary arteries could offer an explanation of the phenomenon

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Quinidine Concentrations in Serum Following Two Different Types of Delayed-absorption Tablets

By

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Sampson et al (10) in 1952 found that quinidine sulfate, administered in freshly prepared delayed absorption coated tablets produced maximum concentrations in blood plasma similar to those obtained with equal doses of uncoated quinidine There was however, a delay in the appearance of the drug in the plasma In 1954 a long acting quinidine sulfate preparation, Systodin was put on the market The coating contained cellulose acetate phthalate The aims for standardization were as follows

1 Resistance to 0 1 N phosphoric acid (pH 1 1) for three hours

2 Solubility in alkaline milieu (phosphate buffer pH 7 6)

3 The tablets should begin to dissolve *after half an hour*

In reality the tablets were completely dissolved during 10—20 minutes This coating was recommended by Couvreur et al (3) in 1958

In a study (7) from 1954 single doses of Systodin were given to fifteen healthy persons and concentrations of quinidine in blood were measured Peak levels were reached in 4—10 hours, averaging 7 33 hours Thirteen patients received the drug twice a day Variations during a 24 hour period in any one patient were insignificant In a study (8) of 40 patients previously converted to sinus rhythm, Systodin was given as maintenance dosage Lack of absorption was seen in 1 out of 68 patients Richardson et al (9) compared serum quinidine concentrations when U S P quinidine sulfate and a delayed absorption quinidine sulfate preparation were given to the same patients They concluded that the delayed absorption tablets gave an adequate blood concentration with a more convenient dosage schedule than did the U S P quinidine

Bellet et al (1) found quinidine sulfate in the form of longacting tablets to be somewhat disappointing and therefore used quinidine gluconate They stated that the last preparation gave effective and sustained plasma levels when given twice daily

Sjogren and Fryklof (11) and Sjogren (12) describe a new type of long acting

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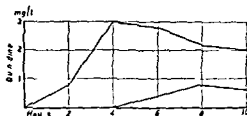


Fig 1 Max and min serum concentrations of quinidine after a single dose of Systodin

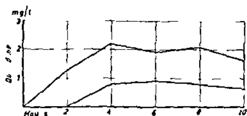


Fig 2 Max and min serum concentrations of quinidine after a single dose of Duretter

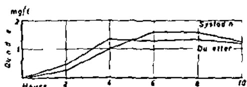


Fig 3 Average serum concentrations of quinidine in 9 patients after single doses of Systodin and of Duretter

preparation, named Duretter In these tablets quinidine bisulfate is dispersed in the pores of a plastic skeleton Cramér et al (4) and Cramér (6) found that compared to quinidine sulfate, Duretter gave significantly lower level of quinidine in serum but that this level remained steady for a significantly longer period The morning concentration of quinidine was the same with 2 doses daily of these tablets as with 4 doses of ordinary quinidine

It therefore seemed to be of some value to compare these two long acting quinidine drugs, Systodin and Duretter, in equivalent doses given to the same patients

Method

The quinidine concentration was determined according to Cramér and Isaksson (5) with use of a Coleman photofluorometer

This method will not give values directly comparable with those obtained in previous studies (7, 8) where the precipitation method of Brodie and Udenfriend (2) was used

Material

Systodin tablets and Duretter, containing equimolecular amounts of quinidine corresponding to 200 mg of quinidine sulfate were used

Nine patients were given Systodin — 0 6 g one day and Duretter in the same dose some days later, or vice versa

Blood samples were drawn 2—4—6—8—10 hours after medication

Results

All 9 patients absorbed both types of drugs

Fig 1 gives the maximum and minimum values with Systodin The values vary considerably from one patient to another, the variation being especially wide the first 6 hours after medication

Fig 2 gives the maximum and minimum values with Duretter Also with this drug there is a considerable variation in the serum concentration of quinidine in different patients

Fig 3 gives the average serum concentrations for both preparations

After a single dose of Duretter the maximum average concentration is reached in four hours, after a single dose

TABLE I Mean values and extreme ranges of quinidine concentration in serum following single doses of Systodin and Duretter in 9 patients

	2	4	6	8	10 hours
Systodin	0 26 mg/e (0—0 8)	1 01 mg/e (0—3)	1 64 mg/e (0 4—2 8)	1 64 mg/e (0 8—2 2)	1 27 mg/e (0 6—2)
Duretter	0 47 mg/e (0—1 3)	1 37 mg/e (0 8—2 2)	1 29 mg/e (0 9—1 9)	1 36 mg/e (0 8—2 1)	1 22 mg/e (0 6—1 6)

of Systodin in 6—8 hours There is little difference between the maximum concentrations following the two preparations These results are also shown in table I

Four of the 9 patients given Systodin did not absorb quinidine within the first 2 hours When the same patients were given Duretter quinidine was absorbed in all but one after 2 hours After 4 hours however, all patients showed quinidine in serum — except for one receiving Systodin

Table II shows the maximum serum concentrations found for Systodin and Duretter in the same patients

Six of the patients had the highest blood concentration ofter Systodin, 2 after Duretter One patient showed the same maximum value after both drugs

TABLE II Maximum concentration of quinidine in serum with Systodin and Duretter in 9 patients

Patients	Systodin	Duretter
K	2 4 mg/e	1 6 mg/e
P	1 4 mg/e	1 2 mg/e
A	2 mg/e	2 2 mg/e
B_1	2 8 mg/e	1 9 mg/e
F	1 6 mg/e	1 2 mg/e
O	1 4 mg/e	1 3 mg/e
D	1 2 mg/e	1 2 mg/e
G	2 3 mg/e	1 8 mg/e
B_2	1 1 mg/e	1 2 mg/e

Systodin seems to produce a slightly higher level than does Duretter

Discussion

There seems to be little difference between these two forms of preparation Systodin is absorbed somewhat more slowly than Duretter with a corresponding delay in attainment of the maximum concentration in serum Especially after 4 to 6 hours Systodin gives considerable more varying values On the other hand,

Summary

A comparison has been made between the effects of Systodin and of Duretter containing equal amounts of quinidine on the serum concentration of quinidine

Quinidine was absorbed in all patients but somewhat slower from Systodin than from Duretter

Maximum values of quinidine were reached with Duretter after 4 hours with Systodin after 6—8 hours In the 4—6 hour period after medication the

values varied more widely with Systodin than with Duretter Maximum values of quinidine in serum seemed to be some what higher after Systodin However, the difference between the two forms of preparations seems insignificant

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Effect of Glucocorticoids Upon the Mucopolysaccharide Content of Human Skin

By

I Lorenzen and H Zachariae

Under physiological conditions glucocorticoids are important factors in the function of the connective tissue Presumably they act upon the cells and on the amorphous intercellular substance as well as the collagen (1, 15 17, 21) The extensive use of these hormones in high, unphysiological doses has given rise to a number of serious side effects which are reflected in the connective tissue As well known examples, the Cushingoid changes of the skin the vascular fragility, and the inhibition of wound healing may be mentioned

Upon administration of high doses of glucocorticoids to experimental animals, the mucopolysaccharide content of the connective tissue has been found to be reduced presumably because of an inhibited synthesis and an increased breakdown (8 21) The mucopolysaccharides are hexosamine-containing polysaccharides which represent a biologically important and characteristic component of the amorphous intercellular substance in the connective tissue (13 21) The skin contains both neutral mucopolysaccharides (5), related to the glycoproteins in the serum (3, 7) and acid mucopolysaccharides (5) cf table I

Animal experiments indicate that the synthesis of collagen too is inhibited during the administration of glucocorticoids in large doses (15)

In 1960 Wright et al (22) reported that they had observed a decrease in the hexasamine collagen ratio in skin biopsies from human subjects after 2 weeks systemic treatment with prednisone This observation was of such fundamental nature that it was felt warranted to repeat, and if possible supplement, the studies

Methods

Skin biopsies were removed with a 6 mm skin punch from the coxal region about 4 cm below the iliac crest from normal looking skin Three biopsies were removed from each patient at intervals of one week The first biopsy was taken alternately from the right and the left side at the start of the

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TABLE I Acid mucopolysaccharides in skin

Non sulphated	Hyaluronic acid
	Chondroitin sulphate A & C
Sulphated	Chondroitin sulphate B

TABLE II Sex age and diagnosis of untreated patients

Case no	Sex	Age	Diagnosis
1	♂	75	Cerebral apoplexy
2	♂	70	Cerebral apoplexy
3	♂	63	Cerebral apoplexy
4	♀	70	Cerebral apoplexy
5	♀	58	Cerebral apoplexy
6	♂	56	Myocardial infarction
7	♀	64	Psoriasis
8	o	64	Psoriasis
9	♂	56	Erysipelas
10	♂	71	Crural ulcer
11	♂	53	Rheumatic fever
12	♂	22	Ankylosing spondylitis
13	♂	70	Gastric ulcer
14	o	68	Chronic pyelonephritis

experiment the second biopsy from the contralateral side and the third biopsy from the same side as the first but about 6 cm away from it The last biopsy was removed at the end of the experiment two weeks after start The biopsies were removed after freezing the skin with chlorethyl and comprised the epidermis as well as the corium The wound was closed with one silk suture Immediately after its removal the biopsy was divided into two halves dried and defatted The total mucopolysaccharide content of the skin was determined by a hexosamine analysis This analysis was done on one half of the biopsy by the method devised by Elson and Morgan (10) as modified by Boas (2) Kirk and Dyrbye (14) and Dyrbye (9) The amount of collagen in the skin was assessed by a determination of the content of the imino acid hydroxyproline

TABLE III Sex age and diagnosis of prednisone-treated patients

Case no	Sex	Age	Diagnosis
1	♀	56	Pustular bacterid of the soles
2	♀	19	Chronic urticaria
3	♂	19	Erythema multiforme
4	♂	26	Allergic contact dermatitis
5	♀	40	Allergic contact dermatitis
6	♂	37	Primary irritant dermatitis
7	♂	62	Primary irritant dermatitis
8	♂	56	Acne conglobata
9	♂	35	Psoriatic arthritis
10	♀	71	Lymphocytic leukemia
11	♂	72	Myelocytic leukemia
12	♂	46	Myelomatosis
13	♂	71	Haemolytic anaemia

which occurs only in collagen (Dempsey and Lansing 1954 (6), Gustavson 1956 (11)) The analysis was carried out by the method of Neuman and Logan (18) in the modification of Martin and Axelrod (16) On the basis of the hexosamine and hydroxyproline analyses the hexosamine hydroxyproline ratio was calculated This ratio is a valid measure of the amount of amorphous intercellular substance in relation to that of collagen fibrils (20)

Material

Two groups of patients were investigated (1) a control group of untreated patients with various cardiovascular and dermatological diseases (table II) and (2) a group of patients with dermatological and haematological diseases (table III) The diseases in the second group were in a chronic active phase such that glucocorticoid therapy was indicated

The treatment period was 2 weeks and the treatment was started immediately after the first biopsy During the first week the patients received 10 mg prednisone 3 times daily and during the second week 5 mg four times daily Prednisone was given orally

TABLE IV Alterations in contents of hexosamine and hydroxyproline and in hexosamine to hydroxyproline ratio in skin from 14 untreated patients

	Hexosamine (μg/mg)[1]	Hydroxyproline (μg/mg)	Ratio
Initial values	3 94±[2]0 15	95 3±4 8	0 042±0 001
Values after one week	4 06±0 24	93 1±4 4	0 044±0 002
Values after two weeks	3 95±0 23	92 2±3 7	0 043±0 002
Changes after one week	0 12±0 19	−2 2±2 9	0 002±0 002
Changes from one to two weeks	−0 11±0 22	−1 0±2 1	−0 001±0 003
Changes after two weeks	0 01±0 20	−3 2±2 2	0 001±0 002

[1] μg/mg dried defatted tissue
[2] Mean ± standard deviation of mean

TABLE V Alterations in contents of hexosamine and hydroxyproline and in hexosamine to hydroxyproline ratio in skin from 13 patients treated for two weeks with prednisone

	Hexosamine (μg/mg)[1]	Hydroxyproline (μg/mg)[1]	Ratio
Initial values	3 93±[2]0 13	99 0±4 9	0 041±0 002
Values after one week	3 85±0 13	101 8±5 6	0 039±0 002
Values after two weeks	3 57±0 10	102 4±5 1	0 036±0 002
Changes after one week	−0 08±0 16	2 9±2 2	−0 002±0 002
Changes from one to two weeks	−0 29±0 16	0 5±3 9	−0 003±0 002
Changer after two weeks	−0 36±[3]0 16	3 4±3 7	−0 005±[4]0 001

[1] μg/mg dried defatted tissue
[2] Mean ± standard deviation of mean
[3] The reduction is statistically significant with p less than 0 05
[4] The reduction is statistically significant with p less than 0 001

Results

From table IV it is apparent that no changes in the hexosamine and hydroxyproline content of the skin or in the hexosamine hydroxyproline ratio occurred in the control group in the course of the 2 weeks The prednisone treated group (table V) showed at the end of 2 weeks a significant fall in hexosamine content and an even more significant fall in the hexosamine hydroxyproline ratio, while the hydroxyproline concentration remained unchanged

Discussion

These results confirm the findings of Wright et al (22) that the mucopolysaccharide collagen ratio in the skin decreases after 2 weeks of prednisone therapy Our studies showed moreover

that this alteration in the ratio is due *to a reduction in the mucopolysaccharide content*

The observations in the control group are not directly comparable with those in the prednisone treated group, as the patients were suffering from entirely different diseases However, it seems *unlikely that the changes in the muco* polysaccharides in the prednisone-treated group could be due to disease activity The analytical results in the control group demonstrated that the mucopolysaccharide collagen ratio in biopsy specimen no 3 was not affected by tissue damage caused by the removal of the first biopsy (12)

The observed reduction in the mucopolysaccharide collagen ratio reflects a reduction in the quantity of amorphous ground substance in relation to the amount of collagen fibrils Thus, it represents a fundamental change of the connective tissue The change in the intercellular substance must be assumed to influence the thriving and function of the cells as the amorphous ground substance is the medium in which the cells are embedded and through which the transport of nutrients and metabolic products takes place (21)

A reduction of the mucopolysaccharide collagen ratio is known to occur in other conditions Sobel and Marmorston (19) and later Clausen (4) demonstrated that a reduction of this ratio is a characteristic change of ageing not only of the skin, but of the connective tissue of several other organs A comparison of the prednisone induced changes in our studies with the changes of ageing demonstrated by Clausen (4) in human skin shows that the changes induced by *two weeks of treatment with prednisone* correspond to the changes normally undergone by skin in the course of about 5 years

Animal experiments indicate that the connective tissue changes caused by glucocorticoids are not restricted to the skin, *but also affect* connective tissue in other parts of the body (20, 21) It is possible, therefore that e g the vascular fragility and the osteoporosis observed after long term glucocorticoid therapy are due to connective tissue changes of this nature

The reduction of the mucopolysaccharide collagen ratio is also characteristic of the increasing fibrosis that may be seen in the connective tissue, for instance during wound healing True, the fall in the ratio in fibrosis is due primarily to an increase in the amount of collagen caused by new formed collagenous fibrils, while the prednisone induced fall in the ratio is due to a reduction in the mucopolysaccharide content In both events, however, the change in the intercellular substance of the connective tissue is of fundamentally the same nature, viz a reduction in the quantity of amorphous intercellular substance in relation to the amount of collagen, a fall in the 'gel fibre ratio (21) It is reasonable *to* assume, therefore, that there is likewise a similarity in the functional *result of* these changes This effect of the glucocorticoids should be taken into consideration in the commonly used long term treatment with these hormones *in* conditions such as collagen diseases and hepatic cirrhosis In these very diseases

fibrosis is an essential morphological component of the terminal stage According to experimental studies, the connective tissue changes induced by glucocorticoid treatment appear to be only partially reversible (20)

Summary

The mucopolysaccharide and collagen content of the skin was determined in skin biopsies from patients on prednisone therapy At the end of 2 weeks a significant reduction in hexosamine content and in the hexosamine collagen ratio was observed reflecting a decrease in the amount of amorphous intercellular substance in relation to collagen fibrils The nature of prednisone induced changes is discussed in relation to the spontaneous changes of ageing in the connective tissue and to the changes seen in fibrosis

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Enlarged Gastric and Duodenal Rugae

The Prognostic Significance of the Radiological Finding of Coarse Mucosal Folds in the Stomach and Duodenum

By

EINAR KRAG

In 1913 and 1923, Forssell (4, 5) described the normal anatomical and radiological appearance of the mucosae of the stomach and duodenum as being a non permanent relief caused by variations in the contraction pattern of the muscularis mucosae Rendich (14) also called attention to the highly variable radiological appearance of the gastric mucosae, both in the same individual at different times and in different individuals He reported that the mucosal folds usually had a width of 2—4 mm (22 in target film distance)

Schwarz (17) Rendich (14) Berg (1) and de Fine Licht (3) regarded a coarse irregular pattern of the gastric mucosae as a characteristic radiological feature of 'hypertrophic gastritis' Folds wider than 5 mm were reported to be pathological In 1934, Kirklin (12) described coarse, irregular duodenal folds as a radiological characteristic of 'duodenitis' It was assumed that the underlying condition of the radiological 'gastritis' or 'duodenitis' was an inflammatory infiltration This concept was supported by the results of histological studies of gastric and duodenal specimens removed at operation

Setala and Siurala (18) and Henning et al (10) contested this concept Setala and Siurala subjected 401 patients to barium meal examination and gastroscopy in 203 of these patients biopsy of the mucosa was also performed Henning et al performed barium meal examination and gastric biopsy in 876 patients In both studies it was concluded that there is no clear-cut relationship between the histological appearance of the gastric mucosa and its radiological pattern Henning et al expressed the view that the radiological diagnosis of 'gastritis' must be abandoned, and said that future studies were required to reveal the pathological significance of well defined radiological changes in the mucosal pattern

Henning (8) Schindler and Templeton (16) and Schatzki (15) showed that in a large number of cases it is not at all

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Table I Survey of diagnoses in the series of 483 patients from 1936—1945

Diagnosis	Coarse irregular mucosal folds		Total
	Present	Absent	
Duodenal ulcer	130	121	251
Gastric ulcer	26	32	58
Pseudo-ulcer	63	111	174
Total	219	264	483

possible to verify by gastroscopy the coarse, irregular folds demonstrated radiologically In addition, Henning (9) reported that there is poor agreement between the diagnoses of gastritis made by gastroscopy and histologically (mucosal biopsy)

At the present time it can only be said that no accord seems to exist among the appearances of the gastroduodenal mucosa revealed by the different methods — barium meals, gastroscopy and histological examination of biopsy specimens The radiological diagnosis of coarse irregular mucosal folds' must stand by itself and cannot be taken as being synonymous with one of gastritis or duodenitis'

Kirklin (12), Ostrow and Resnick (13) and Fraser et al (6) observed typical ulcer dyspepsia in patients with coarse irregular mucosal folds in the duodenum de Fine Licht (3) and Vaughan et al (19) found to some extent, similar symptoms in the presence of these folds in the gastric mucosa

In 1964 Fraser et al (6) performed histamine-infusion tests in a series of 23 patients with coarse irregular folds in the duodenum 80 patients with duodenal ulcer and a control group of 14 normal persons The acid output was of the same range in the first two groups, which both showed hypersecretion as compared with the control group Fraser et al suggested that there is a syndrome characterised by (1) typical ulcer symptoms, (2) coarse irregular mucosal folds, and (3) gastric hypersecretion

Present series

Within the period 1936—1945 inclusive a total of 483 patients were treated in the Department of Medicine Aarhus Amtssygehus for duodenal ulcer gastric ulcer or pseudo-ulcer The patients with pseudo-ulcer showed symptoms of ulcer (syndrôme pylorique) but radiography failed to reveal any lesions

All the patients were subjected to barium meal examination including a study of the mucosal pattern Some of the patients revealed coarse irregular folds in the mucosa of the stomach and/or duodenum (table I)

The diagnoses were made with the same criteria as used by Rendich (14) Berg (1) and de Fine Licht (3) The films were taken in the supine position Folds of a width exceeding about 5 mm were regarded as pathological (*target film distance* about 70 cm 27 5 in see figs 1 and 2



Fig 1 Barium meal supine film Coarse irregular mucosal folds in the stomach

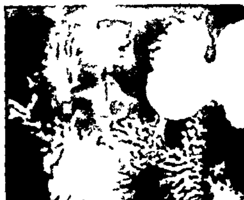


Fig 2 Barium meal supine film Coarse irregular mucosal folds in the duodenum

Follow up examination

A follow up study was made in 1963 Information was obtained concerning 93% of the patients with pseudo-ulcer 99 % of those with duodenal ulcer and 100 % of those with gastric ulcer

All the patients who had died or undergone gastric operation were followed to death or operation while the others were requested to return to the hospital for examination As regards the patients who had died before the follow up examination, information as to the cause of death and the course of the ulcer disease was obtained in each individual case by means of death certificates and through the patient s own doctor The hopsital records for all patients who had been admitted to hospital for medical or surgical treatment of the ulcer disease during the observation period were reviewed

At the follow up examination the patients were divided according to the course of the ulcer disease during the observation period as defined in table II

The association of the severity of the clinical course and the presence or absence of coarse irregular mucosal folds on the first admission in the period 1936—1945 appears from table III It is seen that in patients with duodenal ulcer a poor prognosis

TABLE II Criteria used in defining the clinical course during the observation period

Clinical course	Criteria
A Favourable	Complete freedom of symptoms or only mild dyspepsia not requiring any treatment
B Less favourable	One re admission and or one manifest bleeding and or recurrence causing absence from work
C. Serious	Several re admissions and or manifest bleedings Gastric operation Death from ulcer disease

TABLE III Relationship between mucosal pattern and clinical course

1936—1945 / 1963	Gastric ulcer Coarse irregular folds Present (No)	Present (%)	Absent (No)	Absent (%)
Clinical course				
A Favourable	6	23	13	41
B Less favourable	6	23	8	25
C Serious	14	54	11	34
Total followed up	26	100	32	100
Not followed up	0		0	
Total	26		32	
		$\chi^2 = 2.624$ $f = 2$ $p > 0.1$		

TABLE IV Relationship between mucosal pattern and the frequency of gastric operations during the

1936—1945 / 1963	Gastric ulcer Coarse, irregular folds Present (No)	Present (%)	Absent (No)	Absent (%)
Gastric operation	10	38	4	13
No gastric operation	16	62	28	87
Total followed up	26	100	32	100
		χ^2 5.209 $f = 1$ $0.02 < p < 0.05$		

is significantly related to the occurrence of coarse irregular mucosal folds

It appears from table IV that the frequency of gastric operation is higher in patients with coarse, irregular folds in each of the three groups Gastric operation must be taken as an expression of a serious course of the ulcer disease In 75 % of the cases the indication for operation was solely severe persistent dyspepsia which did not respond to conservative therapy in the remaining cases the indications were of a more complex nature often including retention or haemorrhage

Discussion and conclusions

The series considered here is selected, consisting as it does of patients admitted to hospital with ulcer symptoms The study therefore shows that in these patients the radiological finding of coarse irregular mucosal folds in the

Duodenal ulcer Coarse irregular folds				Pseudo-ulcer Coarse irregular folds			
Present		Absent		Present		Absent	
(No)	(%)	(No)	(%)	(No)	(%)	(No)	(%)
34	26	34	28	24	41	53	52
13	10	28	23	12	21	23	32
82	64	59	49	22	38	26	25
129	100	121	100	58	100	102	100
1		0		5		9	
130		121		63		111	
	$\chi^2 = 9\ 043$, $f = 2$ $0\ 01 < P < 0\ 02$				$\chi^2 = 2\ 824$ $f = 2$ $p > 0\ 1$		

observation period

Duodenal ulcer Coarse irregular folds				Pseudo-ulcer Coarse irregular folds			
Present		Absent		Present		Absent	
(No)	(%)	(No)	(%)	(No)	(%)	(No)	(%)
62	48	36	30	14	24	8	8
67	52	85	70	44	76	94	92
129	100	121	100	58	100	102	100
	$\chi^2 = 8\ 734$ $f = 1$ $0\ 001 < p < 0\ 01$				$\chi^2 = 8\ 200$ $f = 1$ $0\ 001 < p < 0\ 01$		

stomach and/or duodenum seems to be of prognostic importance since it is significantly related to a severer clinical course

The problem is then to what extent is it possible to make an unquestionable radiological diagnosis of coarse irregular mucosal folds on the basis of a barium meal? Is this diagnosis reproducible?

In our hospital all films were assessen by the chief radiologist and only in unquestionable cases were coarse, ird regular mucosal folds recorded Nr studies on the reproducibility of the radiological diagnosis of these folds are available in the literature but Garland 7 estimated that in general errors in radiological diagnosis may occur in up to 30 % of the cases Etter et al (2)

found that 20—30 % of cases with duodenal ulcer were misdiagnosed, while Hornnes and Kinsey (11) reported that the frequency of erroneous diagnoses was 2.4 % in 590 barium meal examinations.

In order to get an indication of the observer error in the radiological diagnosis of "coarse, irregular mucosal folds", the chief radiologist and the author of this paper reviewed 265 barium meal films from patients with dyspepsia. We found 100 with the said diagnosis, while the remainder either had an ulcer or were normal. In a reappraisal of all the films a fortnight later, we found 93 of the 100 in which the coarse, irregular folds had been diagnosed on the first occasion. In the remaining seven the diagnosis was doubtful. This analysis suggests that the radiological diagnosis of the mucosal pattern is subject to an uncertainty of 5—10 %. If allowance is made for a margin of error of ± 5 % in the figures in table IV, the differences disclosed are still significant.

Summary

A total of 483 patients with ulcer-like dyspepsia, including 309 with peptic ulcer, were subjected to barium meal examination with a view to the occurrence of coarse, irregular mucosal folds in the stomach and/or duodenum.

From 17 to 27 years later 96 % of the patients were studied clinically. The frequency of gastric operation was significantly higher in patients in whom these folds were originally revealed. This radiological diagnosis thus seems to foreshadow a poorer prognosis with more symptoms than are seen in patients with a normal mucosal pattern at the first examination.

Acknowledgement

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From the First Medical Service Sahlgrenska sjukhuset University of Goteborg Sweden

Studies on Adipose Tissue from Obese Patients with or without Diabetes Mellitus

III Transformation of U ^{14}C-Acetate and 1-^{14}C Glycerol into Carbon Dioxide and Lipid

By

BERTIL HOOD and PER BJORNTORP

Two distinct types of obesity can occur in the experimental animal on the one hand the regulatory obesity, in which hyperphagia is produced by hypothalamic lesions, and on the other hand the metabolic type in the obese, hyperglycemic mouse With the latter metabolic abnormalities are demonstrable in adipose tissue studied in vitro These include a diminished sensitivity of the lipolytic system to epinephrine stimulation, a lack of ketone body increase during prolonged starvation, increased acetate lipogenesis, and also evidence of glycerokinase activity (10) Some of these features seem to be present also in certain cases of human obesity Thus the lack of increase of ketone bodies during prolonged starvation is a well known phenomenon (8) Increased blood glucose levels are present in obesity with diabetes mellitus It has also been reported that obese humans are less sensitive to epinephrine which a less than normal increase of plasma free fatty acids (FFA) (6) indicating perhaps a decreased sensitivity of adipose tissue to the lipolytic effect of epinephrine Lipogenesis from acetate has been studied in human obesity in a few cases recently (5) and no difference was found as compared with normals Glycerol utilization activity in adipose tissue has not been looked for so far in human adipose tissue in obesity These activities were investigated in the present work in obese patients with or without diabetes mellitus

Material

Both the obese groups six patients without and six patients with diabetes mellitus have been described earlier (2) The control group consisted of six patients two of whom were operated on for abdominal wall hernia and the other four for gall stone In the first two cases the adipose tissue biopsy was taken under local anesthesia and in the others under general anesthesia during operation for the disease mentioned Four in this group were men two women mean age 42 years with a range of 18—64 years They all had blood

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TABLE I Conversion of U-^{14}C-acetate into carbon dioxide and lipids in human subcutaneous adipose tissue in vitro. Mean ± SEM

	Carbon dioxide		Lipid	
	(mμmoles/μg DNA/h)	(mμmoles/10^7 cells/h)	(mμmoles/μg DNA/h)	(mμmoles/10^7 cells/h)
Controls	0.31 ± 0.17	267 ± 90	0.07 ± 0.04	56 ± 31
Obesity	0.18 ± 0.04	289 ± 72	0.02 ± 0.01	32 ± 14
Diabetic obesity	0.10 ± 0.02	131 ± 30	0	0

TABLE II Conversion of 1-^{14}C-glycerol into carbon dioxide and lipids in human subcutaneous adipose tissue in vitro. Mean ± SEM

	Carbon dioxide		Lipid	
	(mμmoles/μg DNA/h)	(mμmoles/10^7 cells/h)	(mμmoles/μg DNA/h)	(mμmoles/10^7 cells/h)
Controls	0.02 ± 0.01	11 ± 6	0.06 ± 0.02	48 ± 16
Obesity	0.03 ± 0.01	27 ± 9	0.01 ± 0.01	9 ± 8
Diabetic obesity	0.02 ± 0.02	37 ± 15	0.05 ± 0.01	60 ± 12

sugar values below 90 mg per 100 ml in the fasting state and were free from serious diseases except that mentioned. All were in caloric balance as judged from their histories and their calory intake was not decreased the day before biopsy, which was performed in the fasting state.

Methods

The biopsy procedure, the handling of the adipose tissue and the incubation vessels have previosly been described (2). The incubation medium consisted of Krebs-Ringer bicarbonate buffer, pH 7.4, with 4 % bovine serum albumin (Armour fraction V, Batch HG 1371) in a volume of 3 ml. Eight flasks were incubated. The first two contained 60 mM sodium acetate, pH 7.4 and U-^{14}C-acetate (The Radiochemical Centre, Amersham, CFA 229), corresponding to approximately 200 000 cpm, and adipose tissue in 25–40 mg pieces to a final weight of about 200 mg. Antother two flasks served as blanks and contained no tissues. Two flasks contained 10 mM glucose, 30 mM sodium acetate, pH 7.4, 3.3 mM glycerol and 1-^{14}C-glycerol (The Radiochemical Centre, Amersham, CFA 47) corresponding to approximately 200 000 cpm. Two more flasks with the same contents except tissue served as blanks.

After 150 minutes of incubation in a Dubnoff type incubator at 37° C, enzymatic activities were stopped by addition of 0.2 ml 1 N sulfuric acid and carbon dioxide and lipid radioactivities were counted as described earlier (1).

Results were expressed as acetate or glycerol converted into carbon dioxide or lipid calculated from counts obtained and from the values for original specific activity in the incubation medium.

Determinations of DNA and cell count have been described earlier (3).

Results

Table I gives the incorporation figures for acetate into carbon dioxide and lipid. Incorporation into carbon dioxide was of the same magnitude on a molar basis as that previously found for glucose (4), and no significant differences were found between groups. Incorporation into lipid was considerably lower than that obtained with glucose (4). Between obesity and control no differences were found, and in none of the obese diabetic patients was any incorporation found.

In table II the results for glycerol incorporation are given. On a molar basis the incorporation found for glycerol was generally about one tenth of that found (4) for glucose. For the incorporation into carbon dioxide no differences were found between different groups while as regards incorporation into lipids a significantly lower value was obtained in the obese group as compared with the diabetic obese group ($p < 0.01$) and a trend of difference was found between the controls and obese group with lower values in the latter ($p < 0.10 > 0.05$). The same results were obtained whether DNA or cell number was used as reference unit.

Discussion

As in previous reports both morphological and chemical measure of cell number in adipose tissue were used as reference units with essentially the same results (3).

Incorporation of acetate label into carbon dioxide was not statistically different between groups. Lipid incorporation was lower than that obtained from glucose, as described in human adipose tissue also by Hamosh et al (7) and by Goldrick and Hirsch (5). Acetate lipogenesis was low, and not statistically different between the control group and the obese group in agreement with a recent report (5) but could not be demonstrated at all in the diabetic tissue. As in all similar measurements, however, the results are difficult to interpret as long as the specific activity of the immediate precursor pool for lipogenesis is not known. A dilution by intracellular pools of acetate, or a possible competition between acetate and fatty acids, the latter shown to be increased in diabetic adipose tissue (2), might have occurred. Lower values for incorporation of acetate label into carbon dioxide in the diabetic group, although not statistically significant, seem to lend some further support to the possibility of isotope dilution. Isotope dilution cannot explain the complete absence of lipogenesis from acetate however. Lipogenesis from 1-^{14}C-glucose is not decreased in diabetic obese patients (4). These two findings seem to indicate diminished fatty acid synthesis with a preserved alpha glycerophosphate formation. This possibility is now tested with analyses of radioactivity incorporations in the glycerol respectively fatty acids of triglycerides.

Clearly no increase in acetate lipogenesis was found in any of the obese groups examined, such as can be demonstrated in the metabolic type of obesity in the hyperglycemic, obese mouse (10).

The finding of a small but significant incorporation of glycerol into lipid

seems to show the presence of a system in human adipose tissue that can transform glycerol into alpha-glycerophosphate, since the latter seems to be the obligate glyceride-glycerol precursor in adipose tissue (12) Alpha-glycerophosphate production from free glycerol could occur by mechanisms such as an active glycerokinase in human adipose tissue, described in the hyperglycemic, obese mouse (11), or by transphosphorylation reactions as described by Margolis and Vaughan (9) in the rat

A smaller amount of glycerol was incorporated into the lipids of the obese group as compared with the diabetic obese group The obese group also showed a trend to lower values in comparison with the controls In these experiments glucose and acetate were present to augment lipogenesis (cf 10) Since it was shown earlier that adipose tissue from obese patients incorporated more glucose into its lipids than for controls or diabetic obese patients (4), it seems possible that alpha-glycerophosphate from glucose might dilute free glycerol, which is activated to alpha glycerophosphate before transformation into glyceride (12), and bring about the results found

Summary

In order to test possible similarities between metabolic type of obesity in the hyperglycemic mouse and the obesity occurring in man incorporation of label from acetate and glycerol into carbon dioxide or lipid was investigated in obese patients with or without diabetes mellitus and compared with a control group This was done using both deoxyribonucleic acid and measurements of fat-cell number as the basis of reference In the diabetic obese group no incorporation of label from acetate into lipid was found In the non-diabetic obese group a smaller incorporation of glycerol label into lipids was found as compared with the other groups The latter might be explained by dilution of precursor pools in lipid synthesis, an assumption supported by earlier data No similarities between the metabolic type of obesity in the mouse and human obesity were found in respect of the measured parameters

Acknowledgement

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The IIIrd International Congress of Nephrology will be held in Washington, D C., September 25—30, 1966 The primary objective of the Congress is to promote the international exchange of scientific information on activities in the basic and clinical sciences relating to the kidney It is anticipated that some 2,500 research workers and physicians representing almost every country in the world will take part in the week of general sessions, symposia, and concurrent sessions of free communications

The Congress is scheduled under the general sponsorship abroad of the International Society of Nephrology and is sponsored in the United States by the Renal Section, Council on Circulation of the American Heart Association

President of the Congress Dr Robert W Berliner of the National Heart Institute

Secretary-General Dr George E Schreiner, Professor of Medicine at Georgetown University Hospital in Washington, D C

The scientific program will open with a plenary session on Sunday evening, September 25 A general session will be held each morning thereafter, followed by concurrent symposia of invited speakers

Advance registration for the Congress is strongly urged, and is required by May 15 1966, from those submitting abstracts and those from abroad who wish to apply for a Congress subsistence allowance of $ 120 For full information and forms for the submission of abstracts, advance registration, and hotel reservations, write to Secretariat, IIIrd International Congress of Nephrology, 9650 Wisconsin Avenue Washington, D C 20014 Cable address Nephrocon, Washington, D C

The Czechoslovak Medical Congress with International Participation on the theme *Clinical physiology and internal medicine* with a Symposium on *Conception and methods of the clinical physiology* will take place in Prague, from August 29 to September 2 1966

Main themes of the Congress Conception of the clinical physiology as human physiology Kinetics of the substances in the organism Correlation between circulation and respiration

Main themes of the Symposium Discussion to the conception of the clinical physiology as human physiology

Physiology and medicine Methodicalness of the human physiology in health and in disease

Congress site Municipal House Prague, Czechoslovakia

Chairman Prof Jiri Syllaba, M D Dr Sc

General Secretary Ass Prof Ota Riedl M D C Sc

Congress Office IVth Medical Clinic Faculty of General Medicine, Charles University, U nemocnice 2 Praha 2 Czechoslovakia

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From the First and Second Medical Departments University of Helsinki, Helsinki Finland

Anticoagulants and Sodi-Pallares Infusion in Acute Myocardial Infarction

By

LAURI AUTIO, JUHA HAKKILA, GOTTFRIED HARTEL and EERO IKKALA

'Truth is the daughter of time and not of authority' — Patients suffering from myocardial infarction have been treated with anticoagulants for nearly twenty years Until 1961 most studies indicated that this treatment had been beneficial Most physicians treat their patients in acute phase at hospital with anticoagulants In general there has been uncertainty only as to how long the treatment should be continued after the acute phase The most radical viewpoint is represented by the Owren school, according to which the treatment should be started as soon as anginal pains appear, that is before infarction, if possible and continued through the life Another viewpoint representing the other extreme has been expressed in the study by Hilden et al (4) which came to the conclusion that anticoagulant treatment is not relevant even in acute myocardial infarction The sceptical opinion is shared also by McMichael and Parry (5) as well as Brown and MacMillan (1) who regarded the inconvenience of the treatment and the risk of hemorrhages involved as too big in relation to the benefit to be expected Recent studies have thus made the usefulness of anticoagulant treatment questionable even in acute myocardial infarction, and at the same time the need for controlled research to clarify the problem has been recognized

In this connection the effect of Sodi Pallares infusion in acute myocardial infarction has also been studied Sodi Pallares et al (9) have used the "polarizing treatment" with low sodium, high water high potassium infusions in various conditions, including acute myocardial infarction

Series and methods

During the time of study, from March 1963 to September 1964 all patients with suspected myocardial infarction attending the Medical Outpatient Department were hospitalized to the First Medical Department if they were born in odd years and to the Second Medical

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TABLE I The present series and its distribution into First (group I) and Second (group II) Medical Departments

				Number of patients							Number of deaths		
		No of pat.	Mean age (yrs)	With previous stenocardia		With previous infarctions		With shock			In the first 24 hrs	In the period 25—72 hrs	After 72 hrs
Group I	♂	125	54	68	54%	19	15%	14	11%		6	2	9
	♀	38	63	25	66%	8	21%	5	13%			3	5
Group II	♂	112	55	60	54%	20	18%	11	10%		6		7
	♀	42	62	23	55%	8	19%	10	24%		4		5
Total	♂	237	55	128	54%	39	17%	25	11%		12	2	16
	♀	80	63	48	60%	16	20%	15	19%		4	3	10
Total		317	59	176	57%	55	19%	40	15%		16	5	26

Department if born in even years Patients dying at the Outpatient Department were excluded as well as patients who were hospitalized to wrong departments

All patients developing ECG-changes typical of myocardial infarction or having in addition to T-changes suggesting subendocardial infarction the typical clinical course with GOT and SR reactions were included into the final series

Some characteristics of the series are given in table I

The patients in the First Medical Department (group I) were treated without anticoagulants.

The patients in the Second Medical Department (group II) received peroral anticoagulant treatment with sodium warfarin (Marevan® Orion) Heparin was not used at all

In both departments patients born in even months received a Sodi Pallares-infusion the first three days of their hospitalization The daily infusion consisted of 500 ml 10 per cent glucose to which 40 mEq potassium and 16 units regular insulin were added The infusion was given at a rate of 30 drops/minute

Patients who died in the first 24 hours are excluded from the series It generally takes 36—72 hours to reach the therapeutic level with peroral anticoagulants and the purpose of this study was explicitly to explain the effect of anticoagulant treatment

Results

The effect of anticoagulant treatment

Of the 296 patients surviving 72 hours, 144 (group II) received anticoagulants The anticoagulant therapy was followed by the Thrombotest method It was aimed at keeping the clotting activity between 5 and 20 per cent This therapeutic level was reached in 95 patients during the first three days, in 26 patients during the fourth and fifth day and in 23 after the fifth day Thereafter the Thrombotest level remained below 20 per cent during the whole hospitalization in 84 patients, 31 patients had one

TABLE II The occurrence of thromboembolic complications and deaths in patients treated with (group II) and without (group I) anticoagulants Only patients surviving 72 hours are included

		No of pat	No of surviving patients with thromboembolism					No of deaths	
			Total		Venous thrombosis	Pulmonary embolism	Arterial embolism		
Group I	♂	117	6	5%	1	2	3	9	8%
	♀	35	8	23%	6	2		5	14%
Total		152	14	9%	7	4	3	14	9%
Group II	♂	106	1	1%		1		7	7%
	♀	38						5	13%
Total		144	1			1		12	8%

TABLE III The occurrence of thromboembolism in autopsies on 10 patients treated with anticoagulants and 9 patients treated without anticoagulants

	No of autopsies	No of thrombo-embolism observations	No of thromboembolism observations		
			Cardiac mural	Pulmonary	Arterial
Group I	10	6	4	2	2
Group II	9	2	2		1

upward deviation and 29 patients two or more deviations

The only bleeding complication was a slight hematuria in a male patient with a Thrombotest value of 5 per cent

The figures for the occurrence of thromboembolic complications and deaths in groups I and II are given in table II

Autopsy was made in 19 of the 26 patients who died The thromboembolic manifestations found in autopsies are given in table III

There was no significant difference between the death rates of groups I and II, but the thromboembolic complications observed both clinically and in autopsies were more frequent in patients who did not receive anticoagulants

The effect of Sodi Pallares infusion

The infusions were not systematically given to every patient born in even months Cardiac insufficiency and shock were held as contraindication Patients who, according to the number of the month they were born should have received infusion, but for some reason did not form a specific group marked with X

The occurrence of cardiac arrhythmias and deaths in patients with and without

and on occurrence of thromboembolic complications in acute myocardial infarction The material consists of 144 patients treated with sodium warfarin (Marevan) and 152 patients whose treatment did not include this In addition altogether 91 patients from the two groups received Sodi Pallares glucose insulin potassium infusions

Mortality was found to be the same in both groups (15 per cent) In the anticoagulant group there was one case of pulmonary infarction, in the reference group there were 14 cases of thromboembolism The anticoagulant group developed no other complications except one case of slight hematuria No definite benefit was observed from the use of Sodi Pallares infusions

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Amino Acids and Free Fatty Acids in Plasma in Diabetes

I The Effect of Insulin on the Arterial Levels

By

A Carlsten, B Hallgren, R Jagenburg, A Svanborg and L Werk[illegible]

The changes in glucose oxidation in diabetes secondarily influence the oxidation and the synthesis of fatty acids as well as the mobilization of fatty acids from the adipose tissue Primary disturbances of the lipid metabolism in diabetes have been searched for but are not yet definitely proved Insulin lowers the free fatty acid (FFA) level in plasma (7, 10) mainly by suppressing the rate of inflow of FFA from the adipose tissue (1) Insulin also influences the composition of fatty acids within this fraction (25)

Protein metabolism is also influenced by the diabetic state (6, 18, 19, 24, 26) Insulin lowers the concentration of the plasma amino acids (20) and stimulates protein synthesis from amino acids This effect seems to be, at least partly independent of the rate of glucose oxidation (17 22, 28)

The aim of the present investigation was to compare the influence of insulin on the free fatty acids and free amino acids in plasma in diabetics and non diabetic controls

Submitted for publication September 30 1965

Materials and methods

Eleven diabetics (3 ♀ and 8 ♂) [illegible] one of them on two occasions [illegible] were between 20 and 60 years [illegible] duration of diabetes from 1 mon[illegible] Two of the patients were [illegible] treatment the other 9 patients [illegible] IU of insulin per day Eight [illegible] were on a diet used for [illegible] hospital This diet compris[illegible] calories per day with about 3[illegible] caloric intake from fat and [illegible] protein and with a high [illegible] rich vegetables but [illegible] milk potatoes and sweet [illegible] were on the carbohydra[illegible] diet previously describ[illegible] diabetic control group [illegible] male volunteers aged [illegible] male patients with [illegible] hypercholesterolemia [illegible] el 318—684 mg/100 [illegible] 36 to 49 years and [illegible] hyperlipemia and [illegible] cholesterol level [illegible] and triglyceride [illegible] plasma), aged [illegible] uals and the pa[illegible] emia or hyper[illegible] Swedish diet

The investig[illegible] morning after [illegible]

TABLE I The effect of insulin on the arterial plasma level of free fatty acids A. Before insulin μM B Decrease after insulin μM. C. Decrease after insulin per cent of the initial level

		Diabetics n = 12 M±SEM	Control n = 9 M±SEM
$C_{1..}$	A	9.2± 2 5	9 7± 1 9
	B	7 7± 2 7	3 1± 1 1
	C	64 6± 11 1	23 5±10 7
C_{14}	A	25 5± 4 9	29 1± 3 5
	B	20 9± 4 9	10 7± 3 4
	C	62 6± 7 5	31 5± 8 7
C_{15}	A	6 2± 1 1	8 3± 0 7
	B	2 3± 1 3	2 5± 0 2
	C	17 3± 21 0	27 3± 6 8
$C_{16\,0}$	A	233 9± 29 9	165 7±26 5
	B	138 7± 26 8	65 3±14 1
	C	52 7± 5 9	36 7± 6 2
$C_{16\,1}$	A	42 5± 4 7	46 7± 4 5
	B	28 6± 4 8	22.8± 5 4
	C	62 3± 5 5	45 5± 9 8
C_{17}	A	19 3± 2 5	15 4± 1 2
	B	11.5± 2 6	5 6± 1 4
	C	53.5± 6 9	33 4± 7 5
$C_{18\,0}$	A	129 6± 17 8	81 6±10 1
	B	67.8± 14 0	22 7± 4.2
	C	47 3± 5 9	28 3± 4 1
$C_{18\,1}$	A	451 7± 48 6	372 0±39 3
	B	299 4± 46 8	162 5±36 3
	C	60 7± 5 5	47 8± 5 7
$C_{18\,2}$	A	143 1± 28 5	72 1±10 7
	B	91 1± 21 8	29 6± 7 5
	C	58 1± 4 9	40.2± 7 1
C_{19}	A	6 1± 1 4	4 8± 0 7
	B	1 4± 0 9	1 2± 0.8
	C	13 0± 18.2	22 4±14 7
C_{20m}	A	35 4± 4 2	22 8± 2 5
	B	23 7± 4 4	6 0± 1 6
	C	57 0± 9 9	25 9± 5.8
C_{20p}	A	8 5± 1 0	8 9± 1 2
	B	2 8± 0 7	0 8± 0 9
	C	37 4± 7 9	7 9±10 1

Table I Cont

		Diabetics n = 12 M±SEM	Control n = 9 M±SEM
C_{22m}	A	15 8± 2 8	11 8± 1.2
	B	5 5± 2 0	1 3± 0 9
	C	31 4± 7 3	11 6± 7 9
C_{22p}	A	9 9± 1 0	8.2± 1 4
	B	3 4± 1 9	1 5± 0 8
	C	22 6± 19 3	16.5± 9 7
Total FFA	A	1 133 4±124 9	854 ±68 5
	B	701.9±112 7	341 ±66.3
	C	57 1± 5 1	38 0± 5 8

twenty IU of crystalline insulin were administered intravenously within 15 minutes to the diabetics and 8—12 IU to the controls In order to avoid advanced hypoglycemia in the non-diabetic controls, the insulin in 3 cases was administered together with 100 ml of 5 per cent glucose In order to get comparable experimental conditions in the diabetics the same amount of glucose was administered together with the insulin in three of these patients This glucose supply did not obviously influence the changes in the plasma levels of fatty acids or amino acids Blood was withdrawn through a catheter in the brachial artery before insulin and 30 minutes after the insulin had been infused

The techniques used for the catheterization of the brachial artery and for blood sampling, chemical and physiological analyses have already been described (2, 4)

Results

The total body oxygen consumption averaged 241 ml per minute before insulin and 246 ml per minute after insulin in the diabetics, compared with 269 and 280 ml per minute in the non diabetic controls The respiratory quo-

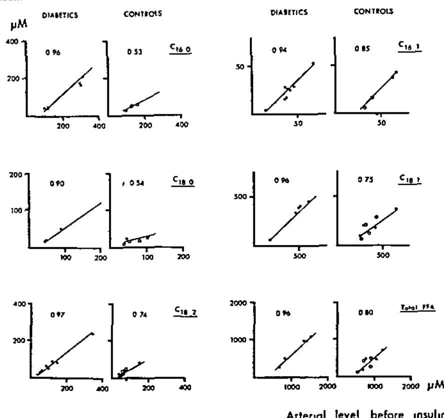


Fig 1 Effect of insulin on the free fatty acids of plasma
○ diabetics ● normals ■ hypercholesterolemics

tient was in the diabetics 0 71 before and 0 81 after insulin, and in the non diabetics 0 81 and 0 91 respectively

The mean ± S D arterial glucose concentration was 332 ± 128 1 mg/100 ml of blood in the diabetics and 104 ± 5 9 mg/100 ml of blood in the controls After insulin the glucose level decreased by 67 ± 40 0 and 42 ± 16 0 mg/100 ml of blood respectively

The pre insulin level of FFA was considerably higher in the diabetics but the composition of the fatty acids within this fraction was rather similar in the two groups (table I) The percentage of linoleic acid was however, higher in the diabetics probably reflecting a higher dietary intake In the diabetics the total FFA level decreased after insulin administration by 702 µM from the initial level of 1,133 µM Among the individual free fatty acids the decrease was not uniform Thus the percentage decrease in stearic acid level was 47 3 per cent and that of oleic acid 60 7 per cent as compared with

TABLE II. The effect of insulin on the arterial plasma level of amino acids. A. Before insulin mg amino acid per 100 ml. B. Decrease after insulin mg amino acid per 100 ml. C. Decrease after insulin per cent of the initial level

		Diabetics n=9	Control n=7	p[1]
		M±SEM	M±SEM	
Threonine	A	1.14±0.134	1.81±0.273	$p<0.05$
	B	0.23±0.061	0.60±0.191	
	C	17 ±6.1	32 ±1.7	$p<0.05$
Proline	A	1.84±0.219	2.57±0.369	
	B	0.46±0.124	0.53±0.136	
	C	23 ±5.9	22 ±6.4	
Glycine	A	1.28±0.117	1.65±0.143	
	B	0.19±0.044	0.29±0.065	
	C	16 ±3.7	17 ±2.9	
Alanine	A	1.63±0.102	2.22±0.180	$p<0.05$
	B	−0.02±0.078	0.10±0.061	
	C	−2.3 ±4.3	2.8 ±2.3	
Valine	A	3.17±0.235	2.45±0.142	$p<0.05$
	B	0.53±0.123	0.28±0.099	
	C	15 ±3.4	11 ±3.1	
Isoleucine	A	1.19±0.147	0.78±0.058	$p<0.05$
	B	0.35±0.074	0.16±0.039	
	C	27 ±3.9	20 ±4.1	
Leucine	A	2.03±0.214	1.41±0.087	$p<0.05$
	B	0.57±0.133	0.30±0.050	
	C	25 ±4.3	21 ±2.2	
Tyrosine	A	0.82±0.074	0.84±0.053	
	B	0.19 0.047	0.18±0.084	
	C	21 ±4.6	21 ±9.1	
Phenylalanine	A	0.74±0.083	0.79±0.013	
	B	0.13±0.013	0.14±0.036	
	C	16 ±3.8	18 ±4.8	

[1] Probability of differences between diabetics and controls arising by chance

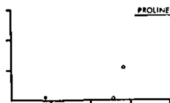
57.1 per cent in total FFA. These changes also caused a significant alteration in the percentage composition of the FFA fraction, the content of stearic acid increasing from 11.4 to 13.7 per cent whereas oleic acid decreased from 40.3 to 35.8 per cent. The difference between the change in percentage of these two fatty acids was highly significant ($p < 0.001$).

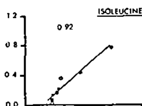
In the non-diabetic controls the total FFA level decreased by 311 μM from the

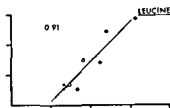
Change after insulin

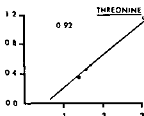
mg / 100 ml

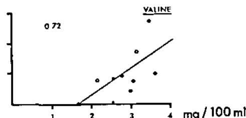












Arterial level before insulin

Fig 2 Effect of insulin on the free amino acids of plasma
○ diabetics ● normals ■ hypercholesterolemics

initial value of 854 μM Among the individual free fatty acids similar changes were observed as in the diabetics The difference between the changes in the percentage of stearic acid and oleic acid was significant ($p < 0\ 01$)

The decrease in free fatty acids induced by insulin was found to be correlated to the pre insulin level (fig 1) There was a proportionally greater decrease at higher arterial levels than at lower ones At the lowest observed levels insulin induced almost no decrease

Before the administration of insulin the mean levels of the branched-chain amino acids valine, isoleucine and leucine were significantly higher in the diabetics than in the controls whereas the levels of threonine and alanine were lower (talbe II)

After insulin administration most of the amino acids decreased markedly both

in the diabetics and in the controls (table II). The alanine level, however, was not depressed by insulin. In 6 of the 9 diabetics there was instead a slight increase of the alanine. For some of the amino acids the decrease induced by insulin was correlated to the pre-insulin level (fig. 2). At the lowest observed amino acid levels insulin had no or a very slight effect. As the correlations in the diabetics and in the controls were similar, the two materials were combined for the calculation of the regression lines. The somewhat greater decrease in valine, isoleucine and leucine and the lower decrease in especially threonine in the diabetics than in the controls might be explained by the differences in the arterial levels of these amino acids.

Discussion

Bierman et al. (1) have shown that the sudden decrease in plasma FFA after insulin is due to a suppression of the mobilization of FFA from fat stores rather than to an increased removal rate of FFA from plasma, as far as can be judged from studies with labelled palmitic acid. Hirsch et al. (13) reported that the percentage of stearic acid is lower in human adipose tissue than in the plasma FFA. If the inflow rate of FFA from the adipose tissue suddenly decreases, as for example after insulin administration, the stearic acid percentage could therefore be expected to increase in plasma FFA simultaneously with the lowering of the inflow of a stearic acid poor fatty acid fraction.

The observed changes in the individual free fatty acids of plasma after insulin could of course also be dependent on an effect of insulin on the efflux of fatty acids from plasma to different organs. As far as we know the removal rates of stearic and oleic acid have never been compared in humans. However, oleic and palmitic acid have been found to have similar rates of turnover and oxidation (9). Rothlin et al. (25) found that the percentage of oleic acid increased linearly with the logarithm of the total FFA level and the percentage of stearic and palmitic acids correspondingly decreased. Our results for healthy individuals (4, 11) and for the present diabetics agree well with the postulates of Rothlin et al. (25) that the composition of the FFA depends on the level in the arterial blood and that at high FFA levels the FFA composition approaches that of depot fat. It seems, therefore, reasonable to assume that the rate of mobilization of FFA from adipose tissue determines the alterations in the fatty acid composition observed in the present study.

A comparison of the effect of insulin on the levels of glucose and fatty acids shows the greatest effect on the FFA level in the diabetics, the level being lowered to a normal post-absorptive range though the glucose level still was enhanced. This observation was likewise made in patients who did not get any glucose supply. The proportionately greater effect on the FFA level fits well with the observations by Zierler and Rabinowitz (29) and Jungas and Ball (16) that insulin inhibits the release of free fatty acids not only via glucose oxidation but also by a direct effect on the lipolysis.

As far as can be judged from the slope of the lines in fig. 1, the insulin effect on FFA seems to be abolished at FFA levels above zero. This observation indicates that at low blood glucose and FFA levels the effect of insulin on the adipose tissue decreases and/or is counterbalanced by the lipolytic activities of other hormones.

In the present study the patients who had a well controlled diabetes showed an increased plasma level of isoleucine, leucine and valine and a tendency to a lowered level of some of the glucogenic amino acids in the morning before insulin was given.

The high level of the branched-chain amino acids, previously found also in experimental diabetes in dogs (14) and in diabetic coma in humans (26), indicates that the diabetic state influences the metabolism of these ketogenic amino acids in a special way. They are structurally closely related and appear to have at least one enzymatic system in common in their degradative pathway (for reference see 15). In contrast with many other amino acids, which are exclusively oxidized by the liver, they are easily oxidized also by non-hepatic tissues (23). In liver perfusion experiments there was a rapid uptake into the liver of all amino acids except isoleucine, leucine and valine, the concentrations of which even increased in the perfusing medium (23). When the perfused livers were taken from alloxan diabetic rats the increase in these amino acids was exaggerated and reached an extreme in the perfusion of the livers taken from ketotic diabetic rats. The increased plasma levels of these amino acids in human diabetes may, therefore, be due at least partly, to an increased liberation from the liver.

When the levels of isoleucine, leucine and valine were rendered normal by the administration of insulin, the levels of the other amino acids became subnormal. It was thus not possible to correct the abnormality in the plasma amino acids completely by insulin. Most well controlled diabetics treated with insulin probably have subnormal levels of many amino acids at least for some time during the day. Whether this is harmful to the organism is not yet proved.

In the present study there was for some of the amino acids a correlation between the arterial levels before insulin and the decrease induced by insulin, without any obvious difference between the diabetics and the controls in this respect. The diagrams illustrating this correlation (fig. 2) might indicate that the concentration of an amino acid cannot be reduced to zero by insulin. This is in agreement with the observation of Harris and Harris (12) that insulin even in doses causing hypoglycemic coma (100—340 IU) in patients with mental diseases does not reduce the total amino acids by more than 33 per cent. In the present study 8—20 IU of insulin caused a decrease in the individual amino acids of about half that size.

To the general rule that insulin lowers the plasma amino acids there was one remarkable exception, viz. alanine, the level of which was not significantly decreased by insulin. In 6 of the 9 diabetics tested there was even a slight increase. This anomalous behav-

iour of alanine has previously been noted by Czyzyk (6), who also found a notably low plasma level of this amino acid in ketotic dogs

Incorporation studies with [^{14}C] alanine into isolated rat-diaphragm protein showed that the incorporation was inhibited by pyruvate and to a lesser extent by glucose even in the presence of insulin (22, 27) This effect was not seen with the other amino acids tested (27) A possible explanation for the peculiar behaviour of alanine was considered to be that the alanine needed for protein synthesis was formed within the cell from pyruvate by transamination (21) This theory fits well with the observations that the plasma alanine is markedly increased during exercise (3) and during the myocardial passage of the blood (2), whereas in the fasting state the intact human liver extracts greater quantities of this amino acid than of any other amino acid even when insulin is given (5) These facts show that there is a formation of alanine in the muscle cells in excess of their need such that there is a flux of alanine from the muscle to the liver The failure of insulin to reduce the plasma level of alanine might be explained by an increased production of this amino acid within the cells such that the alanine requirement for the enhanced protein synthesis is met

Summary

The effect of insulin on individual plasma free fatty acids and free amino acids was studied on 12 occasions in 11 diabetics, and on 9 occasions in a non-diabetic control group including 5 healthy volunteers and 3 patients with essential hereditary hypercholesterolemia and 1 patient with essential hyperlipemia The pre insulin level of FFA was considerably higher in the diabetics but the composition of the fatty acids within this fraction was rather similar in the two groups In both groups insulin administration caused a rapid decrease in the FFA level Among the individual fatty acids the percentage of stearic acid increased and that of oleic acid decreased The decrease in individual free fatty acids induced by insulin was correlated to the pre insulin level Insulin had a greater effect on the level of plasma free fatty acids than on blood glucose In the diabetics insulin normalized the free fatty acid level while the glucose level was considerbly less influenced This observation indicates a direct effect of insulin on lipolysis in adipose tissue

Before insulin the levels of valine isoleucine and leucine were higher in the diabetics whereas the levels of threonine and alanine were lower In both groups insulin decreased most of the amino acids in proportion to the pre-insulin levels The alanine was unchanged, which indicates that insulin stimulates the cellular production of this amino acid

Acknowledgement

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Comparative Studies on Spironolactone (Aldactone) and Chlorthalidone (Hygroton) in the Treatment of Arterial Hypertension

By

Th Friis, J Lintrup and N I Nissen

It is an established fact that diuretics of the sulphamyl group exert an anti hypertensive action in benign hypertension The mechanism of this action is not definitely understood, a number of different factors are operative During the first week of the medication, the increased excretion of sodium gives rise to a decrease in plasma volume and extracellular fluid (9, 30 and others) At the same time the minute volume of the heart is reduced and the peripheral resistance increased (7, 10)

The antihypertensive effect during long term treatment cannot, however be thus explained since the plasma volume and minute volume again increase within 3 or 4 weeks without the blood pressure rising (3, 32 34 and others) During continued treatment infusion of plasma as well as of a sodium chloride solution likewise does not make the blood pressure rise It is believed that now the peripheral resistance has been reduced (3, 17)

It has also been demonstrated that the aldosterone antagonist spironolactone (Aldactone) has an antihypertensive effect (16 29) but the mechanism has not been elucidated In connection with this treatment too Hollander et al (16) found a decreased plasma volume, but only in half of 12 cases of uncomplicated hypertension Infusion of sodium chloride or plasma did not counteract the fall of blood pressure These authors conclude that the hypotensive action of spironolactone is not due exclusively to the natriuretic and possible plasma-volume reducing effects

This raises the possibility that there might be secondary hyperaldosteronism in arterial hypertension which might be imagined to contribute to the elevation of the blood pressure partly by way of a sodium retaining effect and partly by

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sensitizing the vessel wall to pressor agents (15) According to Genest et al (12, 13) benign as well as malignant hypertension is accompanied by increased excretion of aldosterone Laragh et al (19, 20, 21) however, could demonstrate an increased aldosterone secretion rate' only in malignant hypertension and in severe cases of renal arterial stenosis, while in essential benign hypertension the findings were normal Similar results have been obtained by others (4, 6, 18 26, 28, 36)

Only a meagre literature exists on the clinical value of spironolactone in the treatment of hypertension This applies in particular to malignant hypertension and hypertension caused by renal arterial stenosis As far as benign essential hypertension is concerned a larger number of reports have been published (2 11 14, 16 35) On the whole it is reported that spironolactone is frequently of value in the treatment of benign hypertension especially when combined with chlorothiazide derivatives During this type of medication it also counteracts potassium depletion

Cranston et al (5) compared the effect of spironolactone with that of chlorthalidone (Hygroton) in 10 patients with benign hypertension The effect was somewhat inferior to that of chlorthalidone The dosage was 500 mg spironolactone and 50 mg chlorthalidone daily (50 mg chlorthalidone corresponds in antihypertensive effect to 750 mg chlorothiazide) When the two agents were combined, a somewhat stronger effect was obtained A distinct positive correlation was found between the effect of the two drugs

Thus, considering the fairly meagre literature on the value of spironolactone as an antihypertensive agent, we felt justified in assessing its value In doing so, we compared it with a diuretic of the sulphamyl group, viz chlorthalidone (Hygroton), whose antihypertensive value has been established, not least owing to its fairly prolonged action (60 hours) At the same time we evaluated the effect of the two drugs upon the urinary excretion of sodium and potassium

Material and method

The material comprises 5 normotensive subjects without heart disease or manifest oedema 4 hypertensive patients with stenosis of one renal artery confirmed by arteriography and 15 patients with benign hypertension No patient had oedema The patients were kept on a light diet without a supply of salt containing about 80 mEq sodium daily

Investigations and treatment daily determinations of the blood pressure recumbent and erect daily measurement of the 24 hour urinary output and determination of the urinary excretion of sodium and potassium (Eppendorff's flame photometer) On the basis of these tests the average daily excretion of sodium and the average mean blood pressure in each treatment period (mean blood pressure = diastolic pressure + 1/3 of the pulse amplitude) were evaluated Since the recumbent and erect blood pressures differed but little with the drugs used only the recumbent value will be given

The treatment periods were as follows

1 1—2 weeks without treatment

2 2—4 weeks on chlorthalidone (patients admitted on odd dates) or spironolactone (patients admitted on even dates) The doses were large 200 mg chlorthalidone or 225 mg spironolactone daily (the preparation Aldactone (Searle) was used in the micronized form 25 mg corresponds to 100 mg of the

TABLE I Effect of chlorthalidone and spironolactone upon the blood pressure and excretion of sodium in 5 normotensive patients S = Spironolactone C = Chlorthalidone — = No treatment The figures in front of these indices indicate the number of days on treatment

Case no	Sex	Treatment	B P (mm Hg)	Mean pressure (mm Hg)	Sodium excretion (mEq/24 hrs)
1	♀	9—	118/75	89	21
		5 C	102/72	82	108
		6—	95/72	76	20
		14 S	99/69	79	50
2	♂	8—	125/75	92	100
		14 S	110/70	83	137
		7—	111/72	85	182
		15 C	120/82	95	200
3	♂	7—	124/80	95	50
		15 C	130/88	102	63
		13—	133/89	104	31
		14 S	122/88	99	204
4	♂	10—	120/62	81	67
		17 S	106/91	83	109
		5—	105/80	88	100
		13 C	96/69	71	300
5	♀	11—	124/87	99	100
		7 C	133/82	92	129
		5—	120/80	93	150
		14 S	120/80	93	215

original preparation) These doses are sufficient to secure a maximum effect (5 8)

3 1—2 weeks off treatment

4 2—4 weeks on spironolactone or chlorthalidone depending upon the drug given during the 2nd period

5 In some cases a period on combined treatment

In addition the following investigations were performed weekly determinations of serum creatinine serum potassium and serum sodium serum chloride and serum bicarbonate Moreover one weekly determination of blood volume using I^{131} labelled albumin by means of a volemetrone apparatus (Atomium Perkin Elmer) as advocated by Williams and Fine (33) and others (24 27 (dose 3—5 μC I^{131}, normal value 60—95 ml/kg) as well as determination of exchangeable sodium by means of the short lived sodium isotope Na^{24} (about 50 μC) with an equilibration period of 20—24 hours (23) The sodium pool thus determined corresponds to 70—80 % of the total sodium content of the body as it does not include the sodium in the bones Normal values are 39—48 mEq/kg The coefficient of variation for the sodium pool determinations was about 2—5 % and for the blood volume determinations approx 2—4 % In the latter no correction was made for the difference between peripheral and total haematocrits Differences between two determinations less than 15 and 12 percent are not considered significant

Results

Table I gives the results in the 5 normotensive subjects Three were first treated with chlorthalidone 2 with spironolac-

TABLE II Effect of chlorthalidone and spironolactone upon the blood pressure and sodium excretion in 4 patients with stenosis of the renal artery S = Spironolactone C = Chlorthalidone — = No treatment E = Eye-ground The figures in front of these symbols indicate the number of days on treatment

Case no	Sex	Treatment	B.P (mm Hg)	Mean pressure (mm Hg)	E	Sodium excretion (mEq 24 hrs)
1	o	3 —	229/123	158	III	133
		16 S	242/120	165		163
		12 S+C	221/122	155		17[illegible]
2	♂	4 —	196/116	143	II	100
		8 S	205/124	151		108
		21 S	183/124	144		
		13 S+C	176/125	139		
3	♀	6 —	233/126	162	II	67
		13 S	218/125	156		115
		20 S+C	213/125	154		105
4	♀	11 —	224/144	151	II	115
		14 C	198/115	143		134
		7 —	208/113	145		129
		18 S	207/113	145		181
		7 S+C	218/122	154		200

tone Two (cases 1 and 5) showed a slight fall of blood pressure 0—10 mm Hg of the mean pressure; and one case 4) a moderate fall 17 mm Hg of the mean pressure) during the chlorthalidone medication During the spironolactone therapy the blood pressure fell a little in two (cases 2 and 3 The average fall in the mean pressure on chlorthalidone was 2 8 mm Hg and on spironolactone 1 8 mm Hg i e very slight A comparison of the excess sodium excretion on chlorthalidone and spironolactone showed a somewhat greater excretion on chlorthalidone than on spironolactone (average 69 4 and 49 4 mEq daily (tables I and V

The results in the 4 patients with stenosis of the renal artery are shown in table II The eye ground showed changes corresponding to fundus II in 3 and to fundus III in 1 Two exhibited an elevated serum creatinine Of the patients one (case 4) was treated with chlorthalidone alone The effect in this case was slight (fall in mean blood pressure 8 mm Hg) Spironolactone had no cases 1, 2 and 4) or little effect upon the blood pressure case 3 When spironolactone and chlorthalidone were combined there was a slight fall of blood pressure in 3 (cases 1 2 and 3 while case 4 did not respond The average change in mean blood pressure was 2 3 mm Hg on spironolactone The average excess excretion of sodium was in the same range as in normotensive subjects 55 0 mEq daily table V)

TABLE III Effect of chlorthalidone and spironolactone upon the blood pressure and sodium excretion in 8 patients with essential hypertension who failed to respond by a fall in blood pressure to spironolactone S = Spironolactone C = Chlorthalidone — No treatment F = Eye ground The figures in front of these indices indicate the number of days on treatment

Case no	Sex	Treatment	B P (mm Hg)	Mean pressure (mm Hg)	F	Sodium excretion (mEq/24 hrs)
	♀	5—	199/131	154	II	70
		21 S	196/126	149		124
		13 C	180/125	143		130
		60 C+S	166/120	135		
2	♀	7—	213/104	140	II	20
		14 S	209/117	148		57
		4—	208/103	138		75
		10 C	180/96	124		125
3	♂	25—	195/110	138	III	60
		8 C	158/85	109		138
		6—	158/84	109		50
		14 S	176/99	125		165
		8 S+C	166/107	126		145
4	♀	4—	194/116	142	II	50
		25 S	191/112	141		76
		25 C	177/113	134		76
5	♀	17—	222/148	174	III	27
		8 C	227/147	174		53
		4—	207/135	159		38
		8 S	223/146	172		44
		7 S+C	231/146	174		79
6	♀	14—	190/151	164	II	57
		14 S	188/139	155		86
		10—	159/128	138		45
		10 C	159/118	132		75
		43—	157/120	132		47
		15 S	172/123	139		100
		9—	178/125	143		32
		15 C	157/120	132		110
7	♂	14—	197/120	146	II	122
		8 C	176/109	131		200
		6—	188/110	136		70
		15 S	185/108	133		164
8	♂	8—	197/111	140	II	99
		8 C	163/95	118		124
		9—	164/89	114		60
		27 S	166/86	113		98

TABLE IV Effect of chlorthalidone and spironolactone upon the blood pressure and sodium excretion in 7 patients with essential hypertension who responded by a fall in blood pressure in spironolactone S = Spironolactone C = Clorthalidone — = No treatment F = Eye-ground The figures in front of these indices indicate the number of days on treatment

Case no	Sex	Treatment	B P (mm Hg)	Mean pressure (mm Hg)	F	Sodium excretion (mEq/24 hrs)
9	♂	7 —	201/125	150	II	46
		14 S	190/115	140		82
		4 —	183/120	141		50
		9 C	169/110	129		89
10	♀	7 —	202/128	153	II	50
		14 S	183/119	140		86
		15 —	192/120	144		50
		42 C	148/101	117		76
11	♀	15 —	250/136	174	II	53
		14 S	213/121	152		68
		6 —	200/113	142		42
		14 C	188/119	142		75
		9 S+C	176/104	128		78
12	♂	14 —	206/130	155	III	57
		10 S	171/110	140		65
		5 —	158/105	123		50
		9 C	148/98	115		123
13	o	5 —	213/136	162	II	100
		7 S	198/118	145		100
		8 —	193/125	148		56
		7 C	186/114	124		129
14		8 —	227/131	163	III	32
		7 S	202/126	151		91
		5 —	196/113	141		36
		8 C	172/101	125		81
15	♀	12 —	190/107	135	II	50
		15 C	175/105	128		140
		7	190/110	137		79
		14 S	167/95	119		79

This value was not increased by combined medication

The results for the remaining 15 hypertensive patients are recorded in tables III, IV and V, in which the material is grouped according to whether the blood pressure fell on spironolactone alone In 8 there was no or only a negligible fall (fall in mean blood pressure < 10 mm Hg) and in 7 a considerable fall

Table III illustrates the results in cases showing no fall in B P on spirono-

TABLE V Excess excretion of sodium/24 hrs and fall in mean blood pressure in the various groups of patients

	Chlorthalidone		*Spironolactone*	
	Excess excretion of sodium (mEq/24 hrs)	Fall in blood pressure (mm Hg)	Excess excretion of sodium (mEq/24 hrs)	Fall in blood pressure (mm Hg)
1 Normotensive patients (5) (table I)	69 4	2 8	49 4	1 8
2 Patients with stenosis of the renal artery (4) (table II)			55 0	−2 3
3 Patients with essential hypertension (15)	47 5	12 6	38 3	4 5
No fall in blood pressure on spironolactone (8) (table III)	41 2	12 0	50 8	−3 9
Fall in blood pressure on spironolactone (7) (table IV)	54 1	13 4	22 3	15 3

lactone (cases 2, 3, 5 and 6) or only a slight fall (< 10 mm Hg) (cases 1, 4, 6, 7 and 8) Other findings four out of the 8 patients were treated first with chlorthalidone In 3 (cases, 1, 3 and 5) the treatment was combined One patient (case 6) had 2 treatment periods with both agents Six exhibited an eye ground corresponding to fundus II, two to fundus III One (case 5) had an elevated serum creatinine As to the mean blood pressure, it may be seen that this was not reduced by chlorthalidone in one patient (case 5) (in whom it fell however after discontinuation of the drug) In three patients (cases 1 4 and 6) it fell by less than 10 mm Hg, and in five (cases 2, 3, 6 7 and 8) by more than 10 mm Hg On the combined medication the blood pressure showed a further fall in only one patient (case 1) The average decrease on chlorthalidone was 12 0 on spironolactone — 3 9 mm Hg (table V) The excess excretion of sodium was in the same range on spironolactone and on chlorthalidone, the averages being 50 8 and 41 2 mEq/l respectively (table V)

Table IV gives the results in the patients who had a fall in B P of > 10 mm Hg on spironolactone From the table it is evident that only one of the 7 patients received chlorthalidone first In one the treatment was combined (case 11) Five showed eye ground changes corresponding to fundus II, two corresponding to fundus III One (case 12) exhibited elevated serum creatinine The mean blood pressure did not fall at all on chlorthalidone in case 11, < 10 mm Hg in cases 12 and 15 in the other four > 10 *mm Hg On the combined* treatment there was a distinct fall The average fall in blood pressure on chlorthalidone was 13 4 mm Hg on spironolactone 15 3 *mm* Hg *(table V)* The

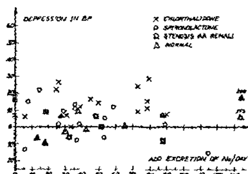


Fig 1 Relation between excess excretion of sodium/24 hours and fall in mean blood pressure

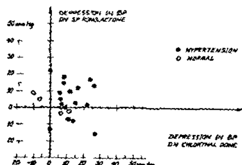


Fig 2 Relation between decreases in mean blood pressure on chlorthalidone and on spironolactone

excess excretion of sodium in this group was somewhat lower on spironolactone than on chlorthalidone The average excess excretion was 34 1 mEq/l on chlorthalidone and 22 3 on spironolactone (table V) It may be mentioned that no correlation was found between the magnitude of the excess sodium excretion and the fall in mean blood pressure This was evident especially (table V) with spironolactone in the patients who did not respond and in those who responded by a fall in blood pressure

Fig 1 sets out the relation between the fall in mean blood pressure and the 24 hour excess excretion of sodium for all the patients It is apparent that no correlation was found, even within the individual therapeutic groups On the whole, chlorthalidone had a somewhat more potent effect than spironolactone as regards the fall in blood pressure (m = = 12 6 and 4 5 mm Hg), without there being a significant difference in the sodium diuresis (m = 47 5 and 38 3 mEq/24 hours)

A comparison of the fall in blood pressure (fig 2) also shows no correlation between the effects of chlorthalidone and spironolactone In 7 the blood pressure fell, as already mentioned >10 mm Hg on spironolactone alone One of these patients gave no response to chlorthalidone and two only a slight response Among patients who responded to chlorthalidone by a fall > 10 mm Hg three showed no response to spironolactone

With regard to blood volume and exchangeable sodium, the following points must be mentioned The blood volume was increased in two of the patients with arterial stenosis Among the remaining hypertensive patients 5 had reduced and one increased blood volume During chlorthalidone treatment of normotensive subjects, the blood volume fell in 4 Among the patients with essential hypertension no definite fall could be demonstrated The patients with stenosis could not be assessed as only one received chlorthalidone alone The action of spironolactone upon the blood volume was a fall in 2 normotensive subjects In the hypertensive patients 2 showed a fall and 2 an increase

Exchangeable sodium was found to be elevated in at least one of the normo

tensive and 3 of the stenotic patients In contrast it was reduced in 5 of the other hypertensive patients, while 2 showed increased values During the treatment the values fell in a total of 4 of the hypertensive patients, 2 on each drug and did not increase in any case

Fig 3 gives the relation between exchangeable sodium and blood volume In this respect there is a slightly positive correlation Exchangeable sodium was, on the average, somewhat lower in the patients with essential hypertension than in the others and they also had a tendency to a lower blood volume

With regard to the serum creatinine it rose over the normal level in 6 patients during chlorthalidone treatment, but in no case during spironolactone treatment The serum potassium fell below normal in 11 of the chlorthalidone treated patients and rose in 4 during spironolactone therapy In the cases when the treatment was combined the tendency to reduced serum potassium was counteracted With regard to the urinary excretion of potassium it was between 20 and 50 mEq/24 hours in almost all patients off treatment and during spironolactone therapy while on chlorthalidone it increased up to 100 mEq/24 hours

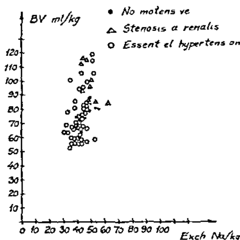


Fig 3 Relation exchangeable sodium and blood volume

Discussion and conclusion

The investigations show that spironolactone had no effect upon the elevation of blood pressure in our 4 patients with *stenosis of the renal artery* According to several authors, this condition, at least in a severe form involves secondary hyperaldosteronism In accordance with this 2 of the 4 patients had hypopotassaemia before the treatment was started On the other hand, there was not a particularly low initial urinary excretion of sodium There was also no major effect upon the blood pressure with combined spironolactone and chlorthalidone

In *essential hypertension* spironolactone had some antihypertensive effect in about half the patients On the whole, however, this effect was weaker than with chlorthalidone This accords the findings of several previous workers We could not, however, demonstrate any correlation between the antihypertensive effect of the two agents as did Cranston et al (5) Among the 4 patients in whom the treatment was combined only 2 gave further response There was no correlation between the antihypertensive effect of the drugs and their sodium-mobilizing effect Thus the degree of the antihypertensive effect of spironolactone is not directly related to its natriuretic action Patients of this category

are not believed to have hyperaldosteronism (4, 19, 20 21), so the antihypertensive action can hardly be attributed to aldosterone antagonism Those hypertensive patients who responded by a fall in B P to spironolactone apparently did not differ from those who did not respond

With regard to the blood volume this was increased in half the patients with arterial stenosis Among the remaining hypertensive patients 5 had reduced and one increased blood volume Biglieri et al (1) found normal and Rochlin et al (25) reduced blood volume in patients with essential hypertension This is in accordance with our results Our findings that the blood volume as a whole did not decrease during chlorthalidone and spironolactone treatment is not in keeping with the findings of several authors using chlorothiazide derivatives (9, 30, and others) but accords with Cranston et al s (5) experience of chlorthalidone Thus, the antihypertensive effect of chlorthalidone and spironolactone in the present series can hardly be attributed merely to a reduction in the blood volume Our finding that exchangeable sodium fell in 4 patients during treatment is not in conformity with other workers (22)

Furthermore it must be emphasized that spironolactone, unlike chlorthalidone, did not entail an increase in serum creatinine in patients with essential hypertension The cause of the increase in serum creatinine on chlorthalidone observed in 6 of the hypertensive patients, is not fully understood

Thus, it must be assumed that the antihypertensive effects of spironolactone is not due, or at least not solely due, to its natriuretic effect and its effect upon the blood volume Here, then, there is reason to raise the possibility of a direct vasodilator effect or an altered sensitivity of the vessel wall to pressor agents

No side effects during spironolactone therapy were observed

Summary

The antihypertensive and natriuretic effects of spironolactone (Aldactone) were studied in a series of 5 normotensive subjects, 4 patients with stenosis of the renal artery, and 15 patients with benign essential hypertension Its effect was compared with that of chlorthalidone (Hygroton) While no effect upon the blood pressure was observed in the normotensive subjects and the patients with renal arterial stenosis, there was a distinct antihypertensive effect upon half the patients with benign hypertension The antihypertensive action was somewhat weaker than that of chlorthalidone There was no correlation between the excess excretion of sodium and the fall in blood pressure, or between the decreases in blood pressure on chlorthalidone and on spironolactone Neither chlorthalidone nor spironolactone exerted any reducing effect upon the blood volume or exchangeable sodium except in a very few of the patients with essential hypertension Between these two parameters there was a slight positive correlation

Acknowledgement

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(Cont from page 354)

The 3rd International Congress of Psychosomatic Medicine will be held in Paris, 15th—18th of September, 1966

Chairman Prof H Desoille

General Secretary Prof P Aboulker

Scientific Secretariat Societe française de Medecine psychosomatique, Hopital Cochin, 27 Rue du Fg Saint Jacques, Paris 14^{e}

Administrative Secretariat Cabinet Henri Dumon, 73 Boulevard Raspail, Paris 6^{e}.

Université de Paris, Faculte de Medecine *Cours de Perfectionnement sur la Nephrologie* les lundi 2, mardi 3 et mercredi 4 mai 1966

Il est recommandé de s'inscrire assez a l'avance, le nombre des participants étant limité Pour tous renseignements s'adresser au secretariat du Professeur Agrege J Crosnier, Hopital Necker, 149 Rue de Sevres, Paris 15^{e}

The Cardio Pulmonary Technicians Association proposes to hold an international meeting during the summer of 1966, the meeting is for technicians engaged in Heart lung technology, Respiratory physiology and Cardiology Meetings have already been held in England for British technicians, and have been found to be very useful and have established new liaison between the various groups throughout the country The association now likes to establish contact with technicians in other countries as it feels that such contacts would be equally valuable

Secretary P A Burgess, Dept of Medicine, Postgraduate Medical School, Ducane Road, London, W 12

The International Conference on Liver Regeneration will be held in Montecatini Terme, Italy, on October 22—23, 1966 under the Chairmanship of Prof Mariano Messini, Director of the Postgraduate School for Liver Diseases, University of Rome

Organizing Committee Milan, Via Modica 6, Italy

Acta Medica Scandinavica Vol 179, fasc 3, 1966

From Medical Department (Head W J Kaipainen, M D), University of Oulu Oulu and Second Medical Department (Head Ilmari Vartiainen M D) University of Helsinki Helsinki, Finland

Effect of Folic Acid on the Absorption of Vitamin B_1 in Fish Tapeworm Carriers

By

J P Palva

The malabsorption of vitamin B_{12} caused by para aminosalicylic acid (PAS) (1) is relieved by folic acid (FA) (5) FA *does not improve the Schilling test* values of patients with deficiency of intrinsic factor and with intestinal malabsorption (5) These findings have led to the assumption that FA plays an important role in the intestinal absorption of vitamin B_{12} This paper deals with the effect of FA on the im paired absorption of vitamin B_{12} in carriers of the fish tapeworm *Dibo thriocephalus latus*

Material and methods

In 12 fish tapeworm carriers with Schilling test (6) values of less than 10 per cent the test was repeated three days later with a *supplement of FA (Folvite® Lederle)* The test dose was 0 7 μg of ^{60}Co B_{12} (Abbott)

To 11 other carriers of the fish tapeworm 2 mg of tritiated folic acid (The Radiochemical Centre) was given on the evening prior to the worm expulsion An alcohol extract of the worm was prepared and the radioactivity of the extract measured in a liquid scintillation counter

Submitted for publication November 10 1965

Results

The results of the Schilling tests performed are presented in table I The mean value rose from 2 7 to 5 7 per cent when a FA supplement was given. The tritiated FA was used to demonstrate *possible FA uptake by the tapeworm* No radioactivity given in this form was found in the worms of the present series, the highest count of the impulses corresponded to less than 0 1 per cent of the test dose

Discussion

In the present series a supplement of FA improved the Schilling test values of the worm carriers just as it did earlier for patients with PAS induced vitamin B_{12} malabsorption (5) It has been found that the tapeworm takes large amounts of vitamin B_1 (2) It could be expected, therefore that it would take FA too But uptake of FA in the worm was not demonstrable The other possibility is that the worm

TABLE I Schilling tests performed ordinarily and with FA supplement

Patient	Schilling test value (%) Ordinary	With FA	Dose of FA (mg)
K L	0 1	0 9	25
E K	0 3	1 2	5
J J	0 6	5 2	25
H P	1 0	1 2	5
S H	1 4	9 5	25
N K	1 5	5 9	5
E J	2 7	1 7	5
A T	3 9	7 8	5
M N	4 2	7 0	5
H S	4 9	2 1	5
P V	5 1	13 0	5
M L	6 9	12 9	5
Mean	2 7	5 7	

excretes material antagonistic to FA This excretion would then, like PAS, impair the absorption of B_{12} through the intestinal wall (5) The haematological remission induced with minimal amounts of FA in a patient suffering from tapeworm pernicious anaemia (4) would thus be explained by an improved absorption of vitamin B_{12} during treatment with FA

The Schilling test values of tapeworm carriers improve immediately after expulsion of the worm (3) A further significant improvement occurs during the following 1 to 6 weeks (3) Thus, although the worm itself has been removed, a disturbance of vitamin B_{12} absorption still remains until restoration occurs in a few weeks

The fish tapeworm may thus have two ways of impairing the B_{12} absorption in its host's intestine The worm takes up about a half of the B_{12} available in the intestine In addition, it disturbs the mechanism by which B_{12} penetrates the intestinal wall, and thus can be relieved by FA

Summary

In 12 carriers of the fish tapeworm the mean value of the Schilling test rose from 2 7 to 5 7 per cent when FA was used as additive The tapeworms of 11 other patients did not take up tritiated FA It is suggested that through an excretion product antagonistic to FA the worm might impair the mechanism needed for the transport of vitamin B_{12} through the intestinal wall

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Acta Medica Scandinavica Vol 179, fasc 4, 1966

From the Departments of Medicine (Head E Ask Upmark M D) and Clinical Physiology (Head G Strom M D of the University Hospital Uppsala Sweden

Effects of Acetylcholine Infusion on the Pulmonary Circulation in Patients with Bronchial Asthma

By

L Irnell and L Nordgren

In a previous study Irnell (8) examined the long term effects of bronchial asthma on respiratory and circulatory function The material was selected from patients who because of an exacerbation of the disease had previously attended hospital and who were re examined during a symptomfree or almost free period The definition of bronchial asthma followed to a large extent the recommendation published by the American Thoracic Society (1) This means that the present material does not include patients with chronic bronchitis Our patients as a group were in a condition that in several respects could be regarded as remarkably good considering that during often great parts of their lives they had been completely or partly disabled by asthmatic symptoms For example all those on whom heart catheterization was performed had normal pressures in the pulmonary circulation at rest With regard to the pressure condition in the pulmonary artery this material differed from series of patients reported earlier from other countries with chronic obstructive pulmonary disease which was found to be accompanied by a tendency to pulmonary hypertension

During physical exercise however the pressure in the pulmonary artery rose to abnormally high values in some of our patients The pressure during exercise was found to have a significant relationship with the estimated intensity of the disease during the years immediately preceding the time of the investigation On the other hand when the overall degree of severity since the onset of the asthma was taken into account the relationship was not significant This was considered to support the view that in cases where the bronchial asthma had been intensive during the last few years it affected the pulmonary circulation even during the symptom free state This influence could thus not be shown during a resting state but only

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TABLE I Schilling tests performed ordinarily and with FA supplement

	Schilling test value (%)		
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K L	0 1	0 9	25
E K	0 3	1 2	5
J J	0 6	5 2	25
H P	1 0	1 2	5
S H	1 4	9 5	25
N K	1 5	5 9	5
E J	2 7	1 7	5
A T	3 9	7 8	5
M N	4 2	7 0	5
H S	4 9	2 1	5
P V	5 1	13 0	5
M L	6 9	12 9	5
Mean	2 7	5 7	

excretes material antagonistic to FA This excretion would then, like PAS, impair the absorption of B_{12} through the intestinal wall (5) The haematological remission induced with minimal amounts of FA in a patient suffering from tapeworm pernicious anaemia (4) would thus be explained by an improved absorption of vitamin B_{12} during treatment with FA

The Schilling test values of tapeworm carriers improve immediately after expulsion of the worm (3) A further significant improvement occurs during the following 1 to 6 weeks (3) Thus although the worm itself has been removed, a disturbance of vitamin B_{12} absorption still remains until restoration occurs in a few weeks

The fish tapeworm may thus have two ways of impairing the B_{12} absorption in its host's intestine The worm takes up about a half of the B_{12} available in the intestine In addition, it disturbs the mechanism by which B_{12} penetrates the intestinal wall, and this can be relieved by FA

Summary

In 12 carriers of the fish tapeworm the mean value of the Schilling test rose from 2 7 to 5 7 per cent when FA was used as additive The tapeworms of 11 other patients did not take up tritiated FA It is suggested that through an excretion product antagonistic to FA the worm might impair the mechanism needed for the transport of vitamin B_{12} through the intestinal wall

References

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5 PALVA I P & HEINIVAARA, O Scand J Haemat In print
6 SCHILLING R F J Lab clin Med *42* 860, 1953

Table I Cont

Patient	AVD (ml/l)		Oxygen uptake (ml/min) STPD		Cardiac output (l/min)		Stroke volume (ml)		Heart rate (beats/min)		Pressures (mm Hg) PA S		PA D		PA M		Br A S		Br A D		Br A M	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
A I B	42 0	46 3	222	199	5 2	4 3	76	63	68	68	23	22	8	9	17	13	120	110	65	70	95	90
F O	56 2	47 4	262	260	4 7	5 5	57	53	82	94	23	15	7	5	14	8	120	108	65	90	90	120
V S	39 6	34 4	214	210	5 4	6 1	69	74	78	82	22	19	9	7	13	11	160	[illegible]	80	90	115	115
M J	42 5	43 3	198	228	4 7	5 3	64	65	74	82	14	14	5	5	8	8	125	120	78	75	95	92
A I S	51 4	48 2	249	211	4 8	4 4	59	53	82	83	16	18	5	6	8	9	135	130	82	80	100	95
K P	46 2	46 4	234	165	5 1	3 6	55	38	92	94	22	17	8	6	13	8	170	155	105	85	130	110
J B N	33 2	39 3	216	193	6 5	4 9	100	65	63	75	24	24	9	11	15	15	110	145	55	70	80	105
M F	52 2	46 5	173	201	3 3	4 3	42	52	79	82	11	10	3	2	5	4	140	135	80	88	110	105
A O	49 9	40 0	201	212	4 0	5 3	61	72	66	74	18	19	8	7	10	12	—	122	—	68	—	92
G C	48 4	40 3	207	228	4 3	5 7	54	63	80	90	18	21	9	9	12	12	205	192	100	92	145	120
K O A	40 2	36 0	293	298	7 3	8 3	96	102	76	81	25	25	12	12	17	15	150	150	85	82	120	115
R M	49 4	41 3	241	190	4 9	4 6	74	62	66	74	24	20	11	7	14	11	180	165	82	82	120	108
V S	40 5	41 0	199	201	4 9	4 8	64	6	76	86	18	18	9	7	13	10	130	118	80	70	100	90
L J	45 9	36 2	189	191	4 1	5 3	41	50	93	105	22	22	11	11	15	15	165	185	95	100	120	140
T H	37 0	40 9	219	234	5 9	6 2	76	68	78	91	14	13	7	7	9	9	140	145	70	80	95	115
A J	57 0	43 3	219	195	3 8	4 5	38	46	100	97	12	14	5	6	8	8	145	105	80	59	100	78
N L	47 8	42 5	206	217	4 3	5 1	64	71	67	72	22	22	10	9	15	13	140	175	95	95	115	130
F L	39 6	38 4	244	322	6 2	8 4	67	91	92	92	31	32	16	17	20	22	125	130	78	75	98	100
E F	34 7	44 1	207	221	6 0	5 0	67	47	90	107	23	20	9	8	14	12	145	—	05	—	115	—
Mean	45 0	41 9	221	221	5 0	5 4	64 4	63	80	86	20 1	19 2	8 5	8 0	12 6	11 3	145	140	82	81	108	107
Highest	57 0	48 2	293	322	7 3	8 4	100	102	100	107	31	32	16	17	20	22	205	192	105	100	145	140
Lowest	33 2	34 4	173	165	3 3	3 6	38	38	65	68	11	10	3	2	5	4	110	105	55	59	80	78

TABLE II The mean figures for differences in certain haemodynamic functions By difference (d) is are also given t equals M/SE For the critical ratio both the total series and the separate

	Oxygen saturation (per cent)		AVD (ml/l)	Oxygen uptake (ml/min) STPD	Cardiac output (l/min)	Stroke volume (ml)
	Br A	PA				
n	19	19	19	19	19	19
M	6 50	5 18	3 05	5 11	−0 33	1 42
SD	4 24	4 15	6 17	28 16	1 04	13 93
SE	0 97	0 95	1 42	6 46	0 24	3 19
t	***6 69	***5 45	*2 15	0 79	−1 37	0 44
t1 (♂ n=8)	**4 40	**3 89	1 79	0 01	−1 85	−0 75
t2 (♀ n=11)	* *4 88	**3 93	1 39	1 15	0 21	1 09

when physical exercise was performed The results indicated that the pressure increase was of a reversible nature

The present report deals with the effects on the pulmonary circulation of acetylcholine infusion in the same series of patients as reported earlier These investigations were considered justified in view of the fact that such studies do not appear to have been made previously in patients with uncomplicated bronchial asthma The effect of acetylcholine infusion upon pulmonary circulation has previously been studied in patients with congenital heart disease (5, 6) mitral stenosis (10 11) primary pulmonary hypertension (9) pulmonary emphysema (2) and even in normal persons (4 7)

Material

Details of this series of patients have been given previously (8) The present investigation was performed on 19 patients selected at random from the main material referred to above

The degree of severity of the disease was assessed by means of so-called sickness points (8) partly for the period since the onset of the disease and partly for the 5-year period immediately preceding the investigation (see table I) The total average number of sickness points was 1 737 and the average number per year during the 5 year period mentioned was 66 According to this evaluation 6 patients had had very severe asthma 10 severe and 3 moderately severe The mean age at onset was 29 years and the mean duration of the disease 22 years

Methods

The methods have been described previously by Irnell (8) The patients received an infusion of acetylcholine into the pulmonary artery the blood pressure being recorded continuously The dose rate was increased by steps until a value of about 11 mg/min was reached Constant infusion of this dose was given for 15 minutes Expiratory air was collected during the 5th to 15th minute of infusion The pressures were measured and

meant C—T (see table I) The standard deviation (SD) standard error (SE) and critical ratio (t) sexes are shown. Degree of significance is denoted by asterisks. For other abbreviations see table I

Heart rate (beats/min)	Pressures (mm Hg) PA			Br A		
	S	D	M	S	D	M
19	19	19	19	17	17	17
−6 26	0 90	0 53	1 32	−2 24	−0 47	0 00
5 13	2 69	1 47	2 21	22 02	11 72	16 61
1 18	0 62	0 34	0 51	5 34	2 84	4 03
** −5 32	1 45	1 56	*2 59	−0 42	−0 17	0 00
* −4 13	1 31	1 67	1 31	−1 22	−0 16	−0 86
** −3 48	0 67	0 86	*2 61	0 53	−0 06	0 77

blood samples taken during the 8th to 12th minute

Right heart catheterization was performed with the usual technique with a double lumen catheter from a left medial cubital vein A polyethylene catheter was introduced into the brachial artery Pressures were recorded in the brachial artery the right ventricle the pulmonary artery and the pulmonary wedge pressure position (PCV pressure) The cardiac output was determined according to the direct Fick principle

The tests were made during a free interval which means that during the period of tests and for at least two days previously the patient was either entirely free from symptoms or in what he regarded as his most symptom free state for the last twelve months On auscultation of the lungs during normal breathing there should either be no rhonchi heard or the above mentioned criterion for the optimal state should apply

Results

As may be seen in table I the degree of hyperinflation — measured as the quotient of the functional residual capacity or residual volume and the total lung volume — varied greatly The mean figures show, however, that these patients regarded as a group exhibited at least a moderate hyperinflation

During the acetylcholine infusion there was an average increase of 6 beats/min in the heart rate and an average reduction of 1 mm Hg in the mean arterial blood pressure The cardiac minute volumes before and during the infusion were also of the same order of size for the material as a whole Previous investigations (2 4, 11) have shown that the "pulmonary wedge pressure is not affected by acetylcholine infusion provided that the systemic arterial pressure is unchanged

The mean pressure in the pulmonary artery decreased from the control mean value of 12 6 mm Hg to an average of 11 3 mm Hg during the acetylcholine infusion In 10 patients a reduced pressure was recorded in 6 it was unchanged and in 3 it was increased The tendency

to some decrease in the pressure in this material is statistically significant (*), as shown in table II. This table which is based on the material presented in table I, gives the difference between the haemodynamic values before and during the acetylcholine infusion.

Significant relationships (*, correlation coefficient $r \pm SE = 0.50 \pm 0.25$) were found between the residual quotient (RV/TLC) and the decrease in the mean pressure in the pulmonary artery during the acetylcholine infusion, a higher degree of hyperinflation usually being accompanied by a relatively large reduction in the pulmonary arterial pressure.

The PCV pressure, which was not measured during the acetylcholine infusion showed ordinary values at rest in all patients. No significant influence on the general circulation was thus noted during the acetylcholine infusion. If it is assumed that the PCV pressure remained unchanged during the infusion, the pulmonary vascular resistance (measured as the difference between the mean pressure in the pulmonary artery and PCV divided by the minute volume) appeared rather to be decreased by the acetylcholine — since some although not a significant increase in the cardiac minute volume occurred — but this change was not statistically significant.

The arterial oxygen saturation which at rest was on an average 95.2 per cent, decreased to 88.7 per cent. This decrease was statistically significant (table II). All patients except one showed a reduction. In this one patient there was an insignificant increase by 0.2 per cent.

Both the oxygen uptake and the stroke volume were unchanged, while the arteriovenous oxygen difference on the average decreased slightly, but with statistical significance (*).

A statistically significant relationship (*, $r \pm SE = 0.52 \pm 0.25$) was found between the reduction in oxygen saturation and the age of the patient at the onset of the bronchial asthma, a high age at onset generally being combined with a large reduction in oxygen saturation. There appeared however, to be no marked relationship between the reduction in oxygen saturation and either the age of the patient, the duration of the disease or the assessed degree of severity of the asthma.

There appeared to be a relatively weak correlation between the decrease in arterial oxygen saturation during the acetylcholine infusion and the maximal ventilation capacity measured as MVV_{40} and MVV_{f} ($r \pm SE = -0.28 \pm 0.25$ and -0.22 ± 0.25, respectively); the correlation with the physical work capacity measured as PWC_{max}, was, however, somewhat stronger, but not significant ($r \pm SE = 0.44 \pm 0.25$). The correlation coefficient between the degree of hyperinflation (RV/TLC) and the decrease in oxygen saturation was 0.31.

Discussion

Analysis of the lung function data indicated at least a moderate degree of hyperinflation in our patients. Further, the maximal ventilation capacity was approximately half that of the predicted value. It can be regarded as probable from these data that this group of

patients exhibited an alteration in the intrapulmonary gas distribution although this was not expressed as any significant increase of the equilibration time for the helium test gas in the lung capacity determinations Disturbance of the gas distribution may mean an uneven perfusion of the pulmonary vascular bed with decrease in the blood flow of poorly ventilated regions For a pressure increase to occur in the pulmonary circulation, a considerable reduction of the perfusional region is required, and it is therefore not necessary that pressure recordings reflect regional changes in the blood flow of the pulmonary vascular bed which are in themselves significant (12 3) In the present patients the resting pressures in the pulmonary artery showed ordinary values and only a very small decrease was seen on acetylcholine infusion A relative constancy of the pulmonary vascular pressure and total resistance is, however, compatible with a regional increase of the perfusion of certain poorly ventilated lung regions and such an effect would explain the observed change in the arterial oxygen saturation from 95 2 per cent at rest to 88 7 per cent under the influence of acetylcholine The result may therefore be interpreted to mean that during acetylcholine 20—25 per cent of the cardiac minute volume was shunted through poorly ventilated lung regions This interpretation means that although the patients had normal resting pressures in the pulmonary circulation, there was a regional effect of the bronchial asthma on the pulmonary circulation (regional has a functional not macroanatomical meaning)

Fritts et al (4) studied the effect of acetylcholine infusion into the pulmonary artery in normal persons Their results as regards pressure conditions in the pulmonary artery were in agreement with those which we found in the asthma patients, but as regards arterial oxygen saturation there was a pronounced difference between our material and that of Fritts et al It is of interest here to compare also the present asthma material with previously reported series of patients with pulmonary emphysema Behnke et al (2) studied the effect of acetylcholine on the haemodynamics in emphysema patients with pulmonary hypertension While the cardiac minute volume and the ventilation remained unchanged, a significant pressure reduction was observed in the pulmonary artery In their material the arterial oxygen saturation at rest was 88 0 per cent, i e considerably reduced decreasing during the acetylcholine infusion to 84 9 per cent The decrease was thus on an average somewhat smaller than that found in the present asthma series

The cardiac minute volume was affected only slightly in the present experiments and with no statistical significance during the acetylcholine infusion Neither were any significant changes noted in the pulmonary or systemic arterial pressures The slight mean increase in the minute volume and the very slight mean decrease in the pulmonary arterial pressure, which were recorded during the infusion may be regarded however as an expression of a tendency to reduction in the pulmonary vascular resistance (the PCV pressure

to some decrease in the pressure in this material is statistically significant (*), as shown in table II This table which is based on the material presented in table I, gives the difference between the haemodynamic values before and during the acetylcholine infusion

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Discussion

Analysis of the lung function data indicated at least a moderate degree of hyperinflation in our patients Further, the maximal ventilation capacity was approximately half that of the predicted value It can be regarded as probable from these data that this group of

choline and the residual quotient This oxygen saturation reduction tended to be larger in cases with relatively low values of physical work capacity and voluntary ventilation capacity, but there was no significant correlation

The results are interpreted to indicate that — in spite of the absence of pulmonary hypertension at rest — there was significant pulmonary vasoconstriction in discrete lung regions The observed signs of reversibility of these vascular changes agree with earlier observed signs of reversibility of ventilatory function (and also of socio medical conditions) *in the present material of patients*

Acknowledgement

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TABLE I Clinical data in 22 *euthyroid* patients treated by radio iodine for tachyarrhythmia

				Before ^{131}I treatment		After ^{131}I treatment					
									Improved[1]		
Case No	Sex	Age	Additional heart disease	No attacks (/month)	T_{24} (%)	Follow up time (years)	No attacks (/month)	Serum cholesterol (mg/100 ml)	Yes	No	Thyroid substitution
1	o	59	—	4	46	9	0		E		
2	♂	58	—	10–12	[2]66	5	1–2	274	E		
3	♂	64	—	1	[2]55	5	1	281		E	
4	♀	77	—	8	45	2	<1	590	H		+
5	♀	30	—	>30	[2]72	7	<1	330	E		
6	o	52	—	10	48	9	[3]0	244	E		
7	♀	61	—	5	36	9	<1	316	E		+
8	♂	40	—	1–2	60	12	<1	393	E		
9	♀	51	—	4–5	55	8	3	295		E	
10	♀	52	—	10	24	4	5	256	E		
11	♂	79	—	30	43	2	<1		E		
12	♀	61	—	>10	[2]45	6	1–2	248	H		
13	o	65	WPW[3]	> 1		1½	0		H		
14	o	65	—	> 1	50	1	<1	250	E		
15	♂	68	CHD	15	47	4	[3]0	282	E		
16	♀	74	—	30	30	1½	30			E	
17	♀	45	RHD	4		10	[3]0	267	E		
18	♂	44	RHD	5		5	0	368	H		+
19	♂	41	VSD[3]	25	22	10	0	250	H		+
20	♀	73	CHD	1	23	4	1	353		H	
21	♂	66	CHD	4	36	4	[3]2–4	152		E	
22	♂	62	CHD	5	33	1½	<1		E		

T_{24} = 24 hours thyroidal uptake of ^{131}I WPW = pre excitation CHD = coronary heart disease
RHD = rheumatic heart disease VSD ventricular septal defect
[1] E = euthyroid H = hypothyroid
[2] Thiouracil treatment withdrawn shortly before ^{131}I tracer test
[3] Perpetual arrhythmia

All patients were disabled by their frequent attacks of tachyarrhythmia and shortly before radio-iodine therapy all had received customary antiarrhythmic medical treatment such as digitalis quinidine or procainamide, at a Department of Internal Medicine with at best temporary relief In five cases an attempt was made to depress the thyroid function by anti thyroid drugs before radio-iodine therapy

The tachyarrhythmia was in all cases paroxysmal Eleven patients had paroxysmal atrial fibrillation nine paroxysmal supraventricular tachycardia and one paroxysmal

atrial flutter One had attacks of ventricular tachycardia In fifteen patients the heart size by roentgen and the resting electrocardiogram were within normal limits Two patients had rheumatic heart disease and one ventricular septal defect Symptoms and signs of coronary heart disease were present in four patients

Methods

In estimating the dose of radio-iodine to be given account was taken of the 24 hour uptake of radio-iodine by the thyroid gland The effective half life of radio-iodine in the gland was at least seven days in all cases The therapeutic dose ranged between 4 and 10 mCi of radio iodine in seventeen patients three patients were given 12 to 15 mCi and two patients more than 15 mCi

After the treatment the patient was followed up at intervals of 2 to 4 months during the first year and then at intervals of 6 to 24 months In eleven patients additional radio-iodine was given 3 to 13 months later All the patients were followed for at least 14 months after the last treatment the longest period being 12 year

The condition of fifteen of the patients reported here is that recorded in the spring of 1965 Two other patients were investigated earlier than 1965 Five patients had died the causes of death being in two patients myocardial infarction and in the others renal carcinoma cerebral hemorrhage and miliary tuberculosis respectively Their condition reported here is that recorded at the last routine examination The mean observation time in the case series was five years

The patient was asked to make a note of the rate and duration of attack of tachycardia He was considered improved if the frequency of attacks had diminished markedly and permanently A reduction in the duration of attacks or an impression that the attacks were less troublesome than before the treatment was not regarded as an improvement

The presence of a hypothyroid state was assessed on the basis of the case history the clinical examination and the laboratory tests including the radio-iodine test and the determination of the protein bound iodine Border line cases were assessed as hypothyroid

Serum cholesterol was analyzed in the Department of Clinical Chemistry at the Karolinska Sjukhuset according to the method described by Pearson et al (14) Samples were obtained in the post prandial state Since the pre treatment values were determined in different laboratories these will not be reported

Results

Effect on tachyarrhythmia Of the twenty two patients treated four were free from attacs Thirteen patients showed striking improvement with the frequency of attacks reduced from one or more a week to a few in a year Several of these patients found the attacks to be less distressing than before the treatment The remaining five patients showed no definite improvement (table I)

Thyroid function During the observation time of 1 to 12 years fifteen patients displayed no clinical or laboratory signs of hypothyroidism (table I) Seven patients had developed hypothyroidism Three of these were of mild degree and did not receive thyroid substitution In the four other the substitution had to be balanced against the frequency of the attacks of tachyarrhythmia and symptoms of hypothyroidism In one case thyroid substitution produced an euthyroid state and in three some degree of hypothyroidism was retained None of the present six hypothyroid patients was greatly distressed by the mild hypothyroid state

Serum cholesterol Serum cholesterol values were obtained in seventeen patients

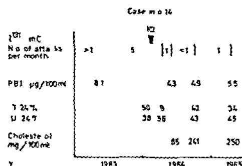


Fig 1 Case no 14 Male born in 1899 No previous heart disease Since 1962 increasing frequency of attacks of paroxysmal fibrillation Quinidine and digitalis gradually less efficient No signs of heart insufficiency Euthyroid Treated with 10 mCi ^{131}I in February 1964 Two months after no attacks of fibrillation despite reduction of the dose of quinidine 14 months after treatment only one single attack of fibrillation

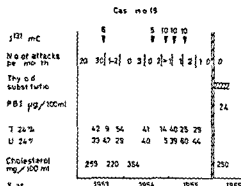


Fig 2 Case no 19 Male born in 1912 Paroxysmal supraventricular tachycardia since his teens 1947 examined for a systolic murmur over the 3rd left interspace 1950 increasing frequency of attacks No effect of quinidine or digitalis Euthyroid. 5 doses of ^{131}I were given (altogether 41 mCi) and the attacks have now disappeared Owing to slight hypothyroidism with hoarseness and a low BMR substitution with 0 02 mg triiodothyronine is given daily

The levels ranged between 152 and 590 mg per 100 ml Values exceeding 300 were recorded in six patients

Case reports In figs 1 and 2 are illustrated the sequence of therapeutic events and the results thereof in two representative patients, of whom one received thyroid substitution for induced hypothyroidism

Discussion

The dose of radio iodine given to the present patient series was on an average relatively large since the normal thyroid gland is less sensitive to irradiation than a hyperfunctioning one and the uptake of radioiodine by the thyroid gland in these euthyroid subjects is lower than for hyperthyroid individuals

It is obviously possible in a number of euthyroid patients to obtain a marked reduction in frequency and severity of attacks by graded depression of the thyroid function without producing even mild hypothyroidism This was so in about one half of the present cases Out of the seventeen patients in whom improvement was recorded, five were hypothyroid In the remaining twelve patients who were improved, depression of the thyroid function without induction of hypothyroidism sufficed to diminish symptoms and frequency of attacks of tachyarrhythmia Of the patients showing no improvement only one had hypothyroidism (table I) It is possible that the therapy could have been rendered more efficient in this group if some degree of hypothyroidism had been accepted The reason why this was not done is that several of these patients claimed that the attacks of tachyarrhythmia were less distressing after the radio iodine treatment

Serum cholesterol measurements were made before and after radio iodine treat

ment, but only those after treatment are reported because of lack of uniformity in analysis before treatment There was no major trend towards marked hypercholesterolemia following radio iodine treatment It has further been pointed out by Blumgart et al in 1955 that even marked hypercholesterolemia, induced by thyroid ablation after radio iodine, does not seem to enhance the incidence of atherogenesis

Since the action of adrenergic substances on the myocardium is dependent on the presence of thyroid hormones (5 15), a reduction in the concentration of these hormones in the blood would be expected to lower the excitability of the heart This may be one major mechanism by which depression of the thyroid function can rid a patient of paroxysmal tachyarrhythmia In cases of hyperthyroidism, tachyarrhythmia is quite common and as is well known, often disappears after the treatment for hyperthyroidism How paroxysmal tachyarrhythmia arises in an euthyroid patient with a seemingly normal heart is not understood In the series of Kurland et al (12) fifteen out of thirty nine euthyroid subjects with paroxysmal tachyarrhythmia had a high uptake of ^{131}I labelled triiodothyronine by the erythrocytes which in conjunction with a normal level of protein bound iodine, points to a reduction in the thyroxine binding capacity of the plasma proteins Since free thyroxine is considered to be the biologically active form of this hormone (6 13 16 17 19) this condition should, however also lead to other symptoms of hyperthyroidism It may be conjectured that the heart is more sensitive than other tissues to variation in the ratio of free to protein bound thyroxine Among the various tissues cardiac muscle has the greatest and earliest calorigenic response to administration of thyroid hormone A selective affinity of the thyroid hormones for the atrioventricular conduction system has also been observed (20)

Summary

Twenty two euthyroid patients with paroxysmal tachyarrhythmia who were resistant to customary anti arrhythmic therapy were treated with radio iodine in doses intended to depress the thyroid function — if possible without production of hypothyroidism The patients were followed for one to twelve years, with a mean observation time of five years

Twelve patients showed marked improvement without signs of hypothyroidism, five were likewise improved while being hypothyroid, and five showed no improvement A possible mechanism by which depression of the thyroid function can rid an euthyroid patient of paroxysmal tachyarrhythmia is discussed

Acknowledgements

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Serum Quinidine Concentration with Two Long-acting Quinidine Preparations

By

I Cullhed, A Hamfelt and E Malers[1]

To maintain sinus rhythm quinidine therapy should be instituted before or immediately after conversion of atrial fibrillation Probably quinidine given before the countershock will reduce the frequency of arrhythmias (6 10, 11) This quinidine therapy should aim at a serum quinidine concentration of between 4 and 7 mg/l in order to prevent recurrence of atrial fibrillation and avoid myocardial toxicity (15 16, 17) Quinidine sulphate is not ideal for this purpose since its rapid absorption necessitates multiple daily doses Several long acting quinidine preparations have been introduced offering a steadier serum quinidine concentration with fewer daily doses

The aim of this study was to compare serum concentrations after a single dose of two long acting quinidine preparations namely Cardioquin® quinidine polygalacturonate, (13) and Kinidin Duretter® (= Quinidine Durules®) quinidine bisulphate embedded in a porous insoluble plastic tablet (5)

Submitted for publication September 3 1965

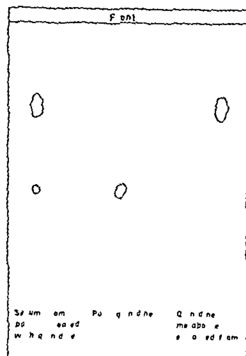
Material and methods

The study was performed on six cases with compensated heart disease who were admitted for conversion of atrial fibrillation Single equivalent doses of the two drugs were given to each case with an interval of as a rule 48 hours The patients were fasting when taking the drug Repeated blood samples were drawn up to 24 hours after the intake

The quinidine concentration in serum was determined according to a method earlier described (1, 9) Quinidine is extracted from serum into an alcohol acetone mixture (95 % ethyl alcohol/acetone p a = 1/1 v/v) In this extract the fluorescence is determined with and without addition of acetic acid and sulphuric acid Because the fluorescence of quinidine does not appear until sulphuric acid has been added the values without the latter take care of any contamination by other fluorescing materials The fluorescence is determined in a 77 200 Beckman Fluorometer

Checking of the method To find out which quinidine components are extracted in this method serum extracts from a patient treated with quinidine were separated with thin layer chromatography on alkaline silica gel G together with as reference substances

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Fg 1 Result of chromatography of serum and for comparison of qu n d ne and a qu n d ne metabol te

TABLE I Recovery of hydroxyqu n d ne

Qu n d es lphate n serum (μg/ml)	Hydroxyqu n d ne added (μg)	Hydroxyqu n d ne recovered (μg)	Reco ery (%)
0	2 5	1 6	70
0	5 0	4 2	84
0	10 0	8 7	87
0	20 0	18 8	94
2 0	2 5	2 8	112
2 0	5 0	5 6	112
2 0	10 0	9 9	99
2 0	20 0	19 8	99
5 0	2 5	2 3	92
5 0	5 0	5 7	114
5 0	10 0	9 6	96
5 0	20 0	18 3	9?
9 9	2 5	2 1	84
9 9	5 0	5 7	114
9 9	10 0	10 4	104
9 9	20 0	17 3	87

pure quin d ne and a qu n dine metabol te (hydroxyqu nid ne k ndly suppl ed by AB Hassle) extracted from urine The chromatography was performed with methanol as sol ent After drying the plates were sprayed with sulphuric ac d (10 % w/v and viewed under ultraviolet l ght to reveal the fluorescence As s shown in fg 1 unmetabol zed qu n d ne and qu n d ne metabolites are included in the extract on

The reco ery of qu n d ne has earl er (9) been reported to be 98 % (range 75—118 The reco ery of hydroxyqu n d ne was accord ng to table I found to be 99 % range 70—114

The coeff c ent of var ation (expressed as per cent of the mean alue was 5 6 calculated from 53 dupl cate determinat ons

Results

In each case the maximum serum concentration was higher after the adminis tration of quinidine polygalacturonate (mean 5 9 mg/l range 3 8—9 2mg/l) than with Kinidin Duretter® (mean 3 8 mg/l range 2 8—5 4 mg/l) The mean 12 hour concentration was for quinidine polygalacturonate 3 0 mg/l (range 1 2—5 6 mg/l) and for Duretter® 2 4 mg/l (range 1 4—3 8 mg/l) After 24 hours the difference in quinidine concentration was as a rule negligible With Duretter® concentrations found at 3 hours were similar to those at 12 hours after the intake

No serious side effects were noted Slight gastro intestinal symptoms oc curred in some patients with both prep arations

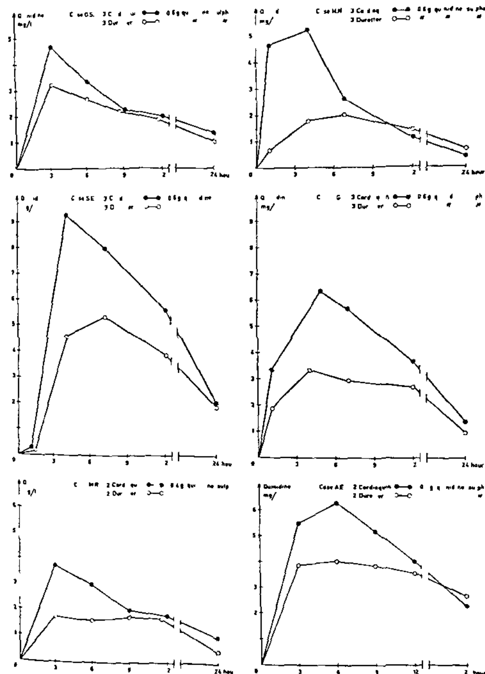


Fig 2 Serum concentrations after single doses of Cardioquin® and Duretter®

Discussion

The method used for serum quinidine analyses was found to be both simple and accurate It is recognized that quinidine concentrations obtained with this method may not be directly comparable with those obtained with other methods The metabolite hydroxy quinidine was found to be included with this method However the therapeutic efficiency of quinidine metabolites has been questioned (3, 4)

The administration of a constant quinidine dose will result in different serum quinidine concentrations in different individuals This applies to maximum concentrations as well as to the time to reach the maximum To some extent these variations persist even if quinidine is given in a dosage related to body weight (5) This has been found also experimentally after intravenous administration (12) Thus any comparison between different quinidine drugs should be performed using one and the same patient group at least if the groups are small In the present study the inter individual differences in quinidine concentration were of the same magnitude for the two preparations These differences could not be explained by differences in degree of heart compensation (2 7)

A comparison of serum concentrations after quinidine sulphate and Kinidin Duretter® has been reported (5) It was shown that due to the sustained release of quinidine from Duretter® there were no early high concentration peaks as is seen after quinidine sulphate the risk of toxic effects being thereby reduced Further a constant serum quinidine concentration could be demonstrated with Kinidin Duretter® given twice daily In a recent paper serum quinidine concentrations were measured after the administration of quinidine sulphate, quinidine gluconate dihydroquinidine gluconate and quinidine polygalacturonate (8) Quinidine gluconate was found to give the lowest early and the highest late serum concentrations during 24 hours after the intake In a comparison between Kinidin Duretter® and a long action quinidine gluconate preparation a more constant quinidine concentration was found with Duretter (14)

In our study, high early serum quinidine concentrations were found after quinidine polygalacturonate but not after Kinidin Duretter® in equivalent doses With the first preparation a concentration in excess of 9 mg/l was found in one patient (Case S E , fig 2) At this concentration the frequency of signs of myocardial toxicity will be significant (17)

Therefore, Kinidin Duretter® seems to be a valuable preparation in long term quinidine maintenance therapy

Summary

A simple method for determination of serum quinidine concentration is briefly described It has been shown that the value by this method represents quinidine together with the metabolite hydroxy quinidine

Two long acting quinidine preparations, Cardioquin® and Kinidin Duretter® were compared with regard to serum concentrations after single equivalent oral doses The mean 12 hour concentration was approximately the

same for the two preparations However, Cardioquin® was absorbed more rapidly, resulting in a considerably higher early serum concentration. With Kinidin Duretter® a steadier serum concentration curve was obtained which should be advantageous in maintenance therapy with quinidine

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Hyponatremia in an Elderly Woman and Inappropriate Secretion of Antidiuretic Hormone

By

JØRN DITZEL[1]

In normal subjects on a high fluid intake the administration of antidiuretic hormone (vasopressin) results in a positive water balance a gain in weight production of urine which is hypertonic to serum hyponatremia and prompt natriuresis All these effects of vasopressin can be prevented by the restriction of water intake (11 12) Disturbances similar to these were first recognized in 1957 by Schwartz and his associates (18) in two patients with bronchogenic carcinoma and they formulated the hypothesis that the hyponatremia and other metabolic abnormalities might be attributable to an inappropriate secretion of antidiuretic hormone (ADH) The arguments for the existence of sustained inappropriate secretion of ADH in these patients have since been greatly strengthened particularly by the demonstration of large amounts of antidiuretic substances in the urine and plasma of such patients (1, 3 13 21) or in the tumor itself (2 3 13) Recently inappropriate release of antidiuretic hormone has also been suggested as the primary cause of hyponatremia and renal salt wasting in acute intermittent porphyria (9 15, 16), in myxedema (8) and in various types of intracranial disease (4, 6 7)

The purpose of the present report is to record the history and the results of a balance study of an elderly woman in whom hyponatremia was observed with excessive urinary losses of sodium, probably resulting from inappropriate secretion of antidiuretic hormone The case differs from most of those previously reported by the apparent absence of any underlying disease process to explain the syndrome with the possible exception of diffuse cerebral vascular disease

Case report

The patient (M O P C 19995) was an eighty six year old woman Apart from an occasional cough and an appendicectomy a few years prior to the admission she had been well until the present illness On November 30th 1963 she was admitted to the Department of Surgery Copenhagen County Hospital Hellerup because of sudden nausea

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and vomiting as well as slight diffuse abdominal pain

Physical examination revealed weight 44 kg blood pressure 130/60 mm Hg maximum body temperature 38 C and pulse 108 per minute She was slightly dehydrated with clouded consciousness Examination of the abdomen revealed no rigidity but tenderness under the right costal arch

Laboratory tests gave the following results erythrocyte sedimentation rate 29 mm in one hour hemoglobin 10 2 g per 100 ml serum sodium 137 mEq/l serum chloride 100 mEq/l serum potassium 3 5 mEq/l total bicarbonate 27 mEq/l serum creatinine 0 7 mg per 100 ml and blood urea 30 mg per 100 ml Urine analyses showed no protein glucose or blood, urine microscopic examination was normal The serum bilirubin was 0 5 mg/100 ml thymol turbidity test 0 03 units glutamic pyruvate transaminase 12 units per ml (normal range less than 40 units) and alkaline phosphatase 8 2 King Armstrong units (normal less than 10 units) The patient was considered to be suffering from acute cholecystitis and was treated conservatively with subcutaneous fluid primarily in the form of 5 per cent glucose solution and gastric lavage The abdominal pain disappeared and her temperature became normal

During the following days hyponatremia was constantly observed despite the administration of large doses of intravenous isotonic sodium solution and the absence of any aspiration or vomiting On December 10th the serum sodium was found to be as low as 113 mEq l Approximately 500 mEq sodium were given intravenously for two days and the serum sodium level increased to 133 mEq/l In spite of this the serum sodium was only 111 mEq/l on the following day (December 14th) Because of this unusual finding the patient was transferred to the Medical Department for further investigation

Here detailed examinations were made of the function of the kidneys the heart the adrenal cortex the liver and adenohypophysis

The physical examination showed as before a thin patient who answered inadequately and was periodically disorientated The temperature was normal The patient appeared normally hydrated and the turgor of the skin and the moisture of the tongue were unremarkable

The patient gave no history of symptoms of kidney disease, repeated urine micro copies were normal and intravenous pyelograms were within normal limits No proteinuria was found Serum creatinine was constantly between 0 7 and 0 9 mg per 100 ml even during periods with severe hyponatremia The maximal urinary osmolality was 610 milliosmols per kg which would indicate that there was no major disturbance of the urinary concentrating mechanism (10) Chromatographic analysis of the urine for amino acids was normal No porphyrinuria was present There was no glycosuria hypophosphatemia hypokalemia acidosis or other signs indicative of tubular damage

No clinical signs of adrenal insufficiency were present The twenty four hours excretion of 17 ketogenic steroids was 5 8 mg which is normal according to the age of the patient A peroral methyrapone test was performed according to the method of Liddle et al (14) and this increased the urinary excretion of 17 ketogenic steroids to 35 4 mg per twenty four hours which is normal and indicative of a normally reacting pituitary and adrenal cortex The twenty four hour output of aldosterone in the urine during large water intake was on day 11 (see below) 4 micrograms (normal range 3—18 micrograms per twenty four hours performed by Dr N Norman Oslo) No physical signs of hypogonadism and hypofunction of the thyroid were found An X ray of the sella turcica was normal

The liver and spleen were not palpable The serum bilirubin was 0 6 mg per 100 ml thymol turbidity test 0 02 units glutamic pyruvate transaminase 24 units per ml and alkaline phosphatase 11 5 King Armstrong units

X ray examination of the thorax showed normal heart size and revealed no evidence of tumour in the lungs or mediastinum The patient had a normal electrocardiogram

and she showed no clinical signs of congestive heart failure The blood pressure was 145/80 mm Hg

For these reasons it was thought that disease of the kidney adrenal and anterior pituitary glands liver lungs and heart could reasonably be excluded as contributory to the present failure to retain sodium

Special studies

At this time it was decided to perform a balance study The study was divided into three intervals (fig 1, table I) During period I (day 1—4) the daily intake of water was restricted to approximately 900 ml in addition to the fluid contained in food During period II (day 5—19) the water intake was augmented to approximately 2000 ml per day During period III (day 20—34) the water intake was again restricted now to approximately 750 ml per day

The patient was placed on a diet containing approximately 24 mEq of sodium per day supplemented with 68 mEq of sodium per day in the form of sodium chloride tablets On day 28 she received an infusion of 340 mEq of sodium *in a 10 per cent solution* (fig 1) and on this day and the following five days *the supplement of sodium chloride* tablets was increased to 136 mEq per day

Apart from an acute weight loss of 1 8 kg during the first three days of water restriction and a *small weight loss on the first days* during fluid restriction in period III the *weight remained* fairly constant throughout the study

The urine was collected for each twenty four hours and the electrolyte and creatinine *contents* of the serum and urine were determined by standard methods As the sodium content of feces is usually quite low and constant (1 to 7 mEq per twenty four hours) despite considerable variation in the excretion of sodium in the urine (5) the fecal excretion of sodium was neglected in the balance calculation Serum and urine osmolalities were determined daily with Fiske osmometer (The osmolality determinations were performed by Dr N A Thorn Copenhagen)

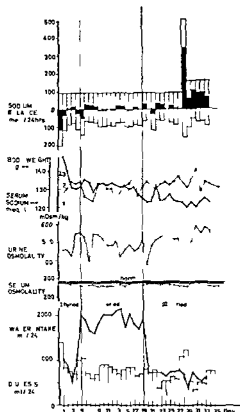


Fig 1 Sodium and water balances during period of study The black columns indicate net sodium balance

The purpose of the metabolic study was (1) to examine the effects of a restricted and an augmented water intake *on the sodium* balance the serum sodium and the urine osmolality during periods with a constant sodium intake (2) to observe the effect of an intravenously administered sodium chloride load upon the sodium balance (3) to examine whether it was possible to produce a maximally dilute urine through a combined load of water and ethanol and (4) to examine the effect of the combined administration of exogenous vasopressin and water on the serum sodium level and urinary sodium excretion

From fig 1 and table I it is seen that throughout the study the presence of hypona

TABLE I Data from metabolic studies on patient M O P C

Day	Body weight (kg)	Intake: Fluid (ml/day)	Intake: Sodium (mEq/day)	Urine: Volume (ml/day)	Urine: Sodium (mEq/day)
Period I					
1	43 8	1 050	92	1 400	203
2	43 0	850	92	850	123
3	42 1	550	92	750	85
4	42 0	1 000	92	700	79
Period II					
5	42 1	2 000	92	1 000	113
6	42 5	1 900	92	1 050	143
7	42 4	1 650	92	700	67
8	41 8	1 600	92	600	71
9	42 3	1 850	92	700	76
10	42 3	2 000	92	900	95
11	42 2	[illegible] 000	92	900	97
12	42 3	2 000	92	850	92
13	42 2	2 100	92	800	96
14	42 1	2 1[illegible]0	92	700	73
15	42 2	1 750	92	800	75
16	42 0	2 050	92	850	97
17	42 1	1 800	92	850	94
18	41 7	1 700	92	650	81
19	41 5	1 900	92	700	93
Period III					
20	41 3	750	92	800	65
21	41 3	750	92	—	—
22	42 2	750	92	800	118
23	41 4	750	92	400	59
24	41 4	650	92	550	64
25	41 7	750	92	550	—
26	41 4	750	92	650	75
27	41 3	800	92	600	88
28	41 3	700	92	1 100	—
29	41 0	400	500	1 250	155
30	41 3	750	160	550	100
31	41 0	600	160	350	51
32	41 3	550	160	525	71
33	41 5	650	160	500	68
34	41 3	—	160	800	93

			Serum			
Chloride (mEq day)	Osmolality (mOsm/kg)	Density	Osmolality (mOsm/kg)	Sodium (mEq l)	Chloride (mEq/l)	Creatinine (mg/100 ml)
193	460	1 006	269	127	87	
117	463	−07	269	130	88	—
84	427	−20	270	134	93	0 7
79	550	−10	276	133	96	0 9
98	555	−09	267	134	96	0 7
149	528	−05	259	126	87	0 7
78	397	−12	266	—	—	—
88	550	−12	267	123	98	0 7
93	548	−12	262	132	93	0 7
124	522	−10	264	135	91	0 7
124	483	−08	258	130	94	0 7
115	482	−06	262	130	94	0 7
109	506	−10	260	124	94	0 7
83	424	−12	270	—	—	—
99	469	−07	264	132	87	0 6
112	492	−10	273	133	95	0 7
107	454	−10	266	137	97	0 7
92	470	07	266	126	95	0 7
98	514	04	268	131	94	0 7
82	377	−06	271	134	97	0 8
—	488	—	275	—	—	—
1[illegible]2	519	14	275	130	96	0 6
62	531	−09	270	133	96	0 7
84	547	−15	275	134	95	0 8
		−10	268	132	97	0 7
95	518	10	266	134	9[illegible]	0 7
85	522	−06	262	137	96	0 7
	406	08	264			—
178		07	260	131	93	0 7
92	508	10	284	—	103	0 8
56	590	−12	288	141	103	0 8
78	553	14	266	129	90	0 8
69	586	12	266	134	94	0 7
111	572	10	266	133	93	0 8

TABLE II Effect on urinary output and urine osmolality of combined ethanol and water load

	Intake		Urine	
Time	Fluid (ml)	Ethanol (g)	Volume (ml)	Osmolality (mOsm/kg)
10 30	—	—	—	592
10 45	200	35	5	582
11 00	100	—	12	610
11 15	100	—	2	488
11 30	100	—	0	—
11 45	100	—	4	582
12 00	100	—	4	588
12 15	100	—	6	608
12 30	100	—	6	586
12 45	100	—	7	590
Total	1 000	35	46	

tremia was associated with a marked loss of urinary sodium varying from 51—203 mEq On the other hand the sodium retaining capacity of the kidneys appeared to be intact On day 28 when the patient was on a restricted fluid intake she received a total of 500 mEq of sodium and approximately 345 mEq could be retained At the same time the serum sodium increased from 131 to 141 mEq per l and the serum osmolality from 260 to 284 mOsm per kg

With fluid restriction during the first four days of the balance study the patient lost 1 8 kg in weight At the same time the serum sodium level increased from 127 to 133 mEq per l and the serum osmolality from 269 to 276 mOsm per kg The increase in serum sodium was probably caused by a reduction of the plasma volume since sodium balance during this period of water restriction continued to remain either negative or only slightly positive

In response to the increased water intake (period II) a slight gain in weight occurred initially At the same time the serum sodium decreased from 134 to 123 mEq per l and the serum osmolality decreased from 276 to 259 mOsm per kg The fall in serum sodium concentration was largely accounted for by dilution since the sodium balance except for the first day was slightly positive During this period the water intake was not followed by an increased output in other words the patient could not properly dispose of the increased fluid intake and the plasma was diluted Later the serum sodium varied from 124 to 137 mEq per l Despite hyponatremia the urinary sodium excretion remained high and the urine was constantly hypertonic compared with serum The initial weight gain subsided probably because the caloric intake of the patient was insufficient for the caloric need due to anorexia

During period III the water restriction was associated with an increase in the serum sodium level from 131 to 137 mEq per l The average serum sodium ($\pm$ S E during this period up to day 28 was 133 $\pm$ 1 6 mEq per l which is significantly higher than the average serum sodium level during period II 130 $\pm$ 1 2 mEq per l ($p < 0 05$ The serum osmolality increased from 268 to 275 mOsm per kg but became normal only after the intravenous infusion of 340 mEq sodium on day 28 During the last five days of the study with the restricted water intake and the relatively large sodium intake there was a clearly positive sodium balance

Throughout the whole study even during periods of marked hyponatremia the serum creatinine remained below 0 8 mg per 100 ml

After the balance study an attempt was made to suppress the endogenous liberation of antidiuretic hormone by the administration of a combined load of water and ethanol The urine was collected in periods of fifteen minutes from 10 30 a m to 12 45 p m through an indwelling catheter Starting at 10 45 a m the patient was given an intravenous infusion of 35 g of ethanol in 200 ml isotonic glucose solution and subsequently 100 ml isotonic glucose solution in each of the following eight fifteen minute periods The total intake of fluid was 1 000 ml Table II shows the urinary outputs and the urinary osmolalities in the respective periods The minimum osmolality was 488 mOsm per kg If it is considered that the ethanol is distributed over a volume equal to 68 per cent of the body weight the concentration in plasma would have been approximately 29 mM Since the concentration in the water phase of the urine is equal to that of the plasma this concentration would contribute to the osmolality of the urine with approximately 29 mOsm per kg Shoen (17) has shown that after intake of quantities of water similar to those employed in this study the urinary osmolality decreased to approximately 60 mOsm per kg and this low value was maintained over two hours If this minimum osmolality of the urine is added to the 29 mOsm per kg an osmolality of approximately 90 mOsm per kg would be expected This theoretical minimum osmolality is considerably lower than the one which was actually found (488 mOsm per kg)

Starting at a time during which the serum sodium level was almost normal the patient was given vasopressin (Insipidin retard[R] 10 i u intramuscularly for 6 days and subsequently 20 i u for 4 days in addition to a fluid load of approximately 3 000 ml per day Fig 2 shows the serum sodium level and the urinary sodium excretion during this study The serum sodium gradually fell from 131 to 112 mEq per l on day 15 When the vasopressin was discontinued the serum sodium

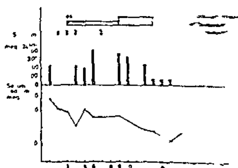


Fig 2 The effect of vasopressin (Insipidin retard[R]) on the serum sodium ... sodium excretion

level gradually increased ... day 21 134 mEq per l ... with the presence of ... sodium excretion in the urine ... 400 mEq per twenty four h...

The following course ... the patient was discharged ... health to the care of her fa... restrict her fluid intake ... per twenty four hours ... patient was readmitted for ... tion Laboratory tests show... results erythrocyte sed... mm in one hour he... 100 ml serum sodium 1... chloride 94 mEq per l ... mEq per l total serum ... per l serum creatinine ... She was feeling well ... an old people's home ... reported that the pat... seven was still act... good health

Comments

The hyponatrem... differed from th... depletion states ... intestinal adren...

found, and signs of hypovolemia, such as hypotension, poor skin turgor and azotemia were absent The case could also be distinguished from the hyponatremia due to congestive heart failure or cirrhosis of the liver by the absence of manifest edema Further in those edematous states the hyponatremia is associated with a minimal urinary loss of sodium and not as in this case with marked natriuresis

The case of hyponatremia under discussion appears to be best explained by inappropriate secretion of antidiuretic hormone

The secretion of the antidiuretic hormone (ADH) is primarily related to the serum osmolality Normally, an increase in serum osmolality will stimulate the release of ADH, which in turn leads to water resorption by the kidney and the formation of a hypertonic urine A decrease in serum osmolality inhibits the ADH secretion, which leads to increased excretion of water and a hypotonic urine Of less importance to the regulation of ADH secretion is the plasma and/or extracellular fluid volume A contracted volume will tend to stimulate ADH secretion, whereas an expanded volume tends to inhibit its release In addition ethanol specifically inhibits the secretion of ADH (20)

The arguments for the existence of sustained inappropriate secretion of ADH in the present case are supported by the following findings firstly the presence of hyponatremia and hypotonicity of the plasma in association with urine hypertonic to plasma secondly a urinary excretion of substantial amounts of sodium despite the presence of hyponatremia and a normal renal conservation of sodium during the period of restricted water intake (Period III), thirdly improvement of the hyponatremia by fluid restriction, whereas an abnormal tendency to water retention and evidence of dilution hyponatremia were present during the period of increased water intake (Period II), fourthly, a urine osmolality that did not decrease significantly during the experiment with a combined load of water and ethanol (table II)

These findings resemble the hyponatremic syndrome studied by Leaf and his coworkers (12) who gave Pitressin® to normal individuals during high fluid intake These subjects retained water and gained weight, and a dilution hyponatremia developed On the third day there was a marked increase in urine sodium excretion, possibly in order to protect the body against extreme increases in fluid volume The sodium excretion however served furthermore to decrease the osmolalities of the body fluids Thus, it appeared that constancy of volume was preserved at the expense of tonicity Both hyponatremia and renal loss of sodium could be corrected by restricting water intake without discontinuing the pitressin administration

The mechanism leading to the increase in sodium excretion is not clearly understood It has been suggested that an increase in the glomerular filtration rate related to the extracellular fluid expansion contributes to the increased sodium loss (4 18 19) Other investigators have maintained that the volume expansion may decrease aldosterone secretion and reduce the renal tubular reabsorption of

sodium As in the present case a low normal urinary excretion of aldosterone has been observed in such patients during periods of high fluid intake and hyponatremia (15, 18, 22) Thus, the expansion of extracellular volume as a result of water retention could augment sodium excretion both by raising the glomerular filtration rate and by suppressing the secretion of aldosterone

The mechanism responsible for the inappropriate secretion of ADH in the present case is unknown Because of the age of the patient, her disorientation and confusion (at times also when the serum sodium was normal) it was conceivable that she suffered from diffuse cerebro-vascular disease The positive stimulus for ADH secretion might therefore be an irritative lesion in the central nervous system The release of ADH could not be significantly inhibited by rapid administration of ethanol and water On the other hand the response of the patient to parenterally administered vasopressin (fig 2) appeared to be normal, and this seems incompatible with the presence of completely autonomous ADH secretion This limited evidence suggests that the faulty control of ADH secretion may be related to defects in an inhibitory phase of ADH release

The prognosis in the present case appeared to be good, when the patient was on fluid restriction (less than 800 ml per day) It is possible that the hyponatremic syndrome only occurs sporadically The good prognosis is in contrast to many previously reported cases of the syndrome in which definite and usually ominous diseases have coexisted

Summary

The history and the results of balance studies in an elderly woman with a low serum sodium and an excessive urinary loss of sodium are reported The primary cause of this unusual case of hyponatremia appeared to be water retention secondary to inappropriate secretion of antidiuretic hormone, possibly resulting from diffuse cerebro vascular disease

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Determination of the Plasma Tyrosine in Thyroid Disorders

A New Test of Thyroid Function

By

K. Siersbæk-Nielsen

In 1961 Sos et al (15) reported that during investigations into the relationship between thyroid function and the metabolism of tyrosine they had found that the plasma tyrosine concentration was increased in patients with thyrotoxicosis Levine et al (7) had, quite independently, made the same observation Both these groups of workers have since published a number of reports of experimental investigations into the tyrosine metabolism in thyroid disease (9 11 12 13 14)

These investigations have not revealed any changes in the enzyme systems of the liver which could account for the alterations in the tyrosine concentration in the plasma and the cause of the changes in plasma tyrosine level which are found in hypothyroidism and hyperthyroidism are as yet unclarified

The aim of the present study has been to assess the value of the determination of plasma tyrosine concentration in the diagnosis of thyroid disease

Submitted for publication September 14 1965

Material and methods

The material comprised 118 patients 15 men and 103 women The control group comprised 39 subjects (9 men and 30 women) whose ages ranged from 14 to 78 years Most of these were patients who were considered to be suffering from functional complaints In addition a group of healthy subjects (nurses and students) have been included in this material

The group of patients with hyperthyroidism comprised 32 women and 4 men aged between 18 and 74 years (fig 1) All patients were untreated at the time of the investigation Two of the patients had previously undergone thyroidectomy 25 had diffuse and 8 had nodular goitre and 6 had exophthalmus Seven had previously received medical treatment but the symptoms had recurred — two of these patients had developed recurrences after treatment with radio-iodine

The patients with hypothyroidism comprised 13 women and 1 man whose ages ranged from 38 to 83 years (fig 1) Five of these patients had previously undergone thyroidectomy and one had received ^{131}I therapy Three of the patients had exophthalmus and one had goitre

Eight patients with diffuse atoxic goitre and 10 with nodular atoxic goitre — all of whom were women — were also investigated

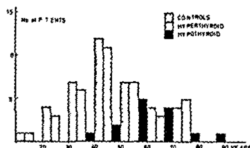


Fig 1 Age distribution of 39 euthyroid 36 hyper thyroid and 14 hypothyroid patients

Blood samples were taken in the morning after the patient had fasted for 10 hours Heparin blood was used this was centrifuged as soon as possible and immediately after centrifugation the plasma samples were placed in either the refrigerator or the deep freezer In the oral tolerance tests a dose of 50 mg tyrosine per kg body weight suspended in about 50 ml water was used The patient fasted throughout the test Blood samples were taken after 30 mins 1 1/2 3 and 4 1/2 hours The intravenous tolerance tests were also carried out in fasting patients Blood samples were taken 5 mins 10 mins 30 mins 1 1/2 3 and 4 1/2 hours after the intra venous injection of 1 g tyrosine in 20 ml aqueous alkaline solution

The fluorometric method described by Waalkes and Udenfriend (16) was used for the tyrosine analyses All determinations were made in duplicate and standard samples were included in each set An Aminco Bowman spectrofluorometer was used The fluorescence of the tyrosine derivates was measured at 560 nanometers using an excitation wavelength of 460 nanometers The final aqueous phase of samples of high con centration was diluted with a solution of the same composition as the blind

Error of measurements On 23 analyses of the same sample of plasma over a period of 6 weeks the tyrosine content was found to be 15 0 ± 0 8 (mean ± 2 S D) µg/ml Each of the results was as stated above the average of 2 determinations

The ^{131}I uptake of the thyroid gland was measured as described by Friis and Korsgaard Christensen (2) The normal range for the 4 hour uptake in the gland is 15—45 % and for the 24 hour uptake 30—70 % The protein bound radio-active iodine expressed as a percentage of the dose per litre serum is normally less than 0 3 The resin tri iodothyronine ^{131}I test (Triosorb Abbott) was carried out as described by Molholm Hansen and Buhl Jorgensen (10) The normal range is 22 3—34 4 %

Results

Fasting plasma tyrosine

Control group

The fasting plasma tyrosine level was found to be 11 3 ± 3 6 µg/ml (mean ± 2 S D) This value is in close agreement with those which have previously been reported (9) Iob et al (4) found that over the course of three months there were considerable variations in the concentrations of a number of amino acids (including tyrosine) in the same patient We have carried out a pilot study of 5 healthy subjects over a period of 4—5 months but have not found a variation of more than 15 % between the lowest and highest level of plasma tyrosine as measured in 4—5 samples from the same subject

Hyperthyroid patients

The fasting plasma tyrosine in this group of 36 patients averaged 18 8 ± 6 2 µg/ml (mean ± 2 S D) This average is 66 % higher than that of the control group and this increase is significant ($p < 0.001$)

It can be seen from fig 2 that there is no correlation between the fasting plasma tyrosine and the basal metabolic rate, resin tri iodothyronine test, protein

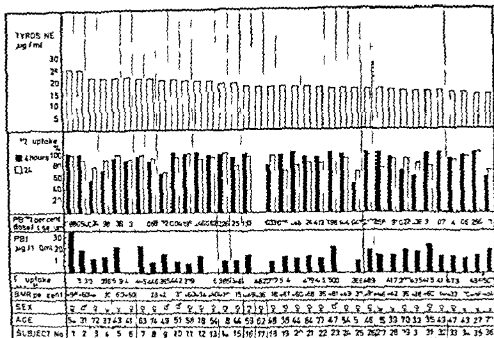


Fig 2 Results of fasting plasma tyrosine determinations and other thyroid function tests in 36 hyperthyroid patients

bound iodine, ^{131}I uptake of the thyroid gland, or the protein bound radio-active iodine No significant variation in plasma tyrosine with age or sex has been found in the group There is *no* significant difference between the average values for patients with and for those without exophthalmus Five of the 36 patients with hyperthyroidism (14 %) had plasma tyrosine levels which lay within the normal range

Hypothyroid patients

The average fasting plasma tyrosine level in this group of 14 patients was found to be 9.1 ± 3.2 µg ml (mean $\pm$ 2 S D This value is 19 % lower than the average for the control group and this decrease is significant ($p < 0.001$) In this group of patients as in those with hyperthyroidism there is no correlation between the fasting plasma tyrosine and the basal metabolic rate protein bound iodine or ^{131}I uptake of the thyroid gland (fig 3 No definite variation with age was found within the group Ten of the 14 patients with hypothyroidism 71 % had fasting plasma tyrosine levels which lay within the normal range Fig 4 shows the fasting plasma tyrosine levels in the patients with hyperthyroidism and hypothyroidism together with those of the control group

Other thyroid disorders

The average level of the fasting plasma tyrosine was found to be 11.3 ± 3.6 µg ml (mean $\pm$ 2 S D) in the patients with diffuse atoxic goitre and that of the

SUBJECT No	1	2	3	4	5	6	7	8	9	10	11	12	13	14
PB ¹³¹I per cent dose/l serum	0 6	0 02	0 08	0 05	0 08		0 05	0 03	0 02		0 03		0 14	0 05
T³ uptake	21 6	23 1	22 6	19 1	22 4					25				22 6
BMR per cent	9	9	6		13	35	12	30	15		22	21	6	25
SEX	♀	♀	♀	♀	♀	♀	♀	♂	♀	♀	♀	♀	♀	♀
AGE	41	60	67	38	52	83	61	56	50	69	43	56	50	70

Fig 3 Results of fasting plasma tyrosine determinations and other thyroid function tests in 14 hypothyroid patients

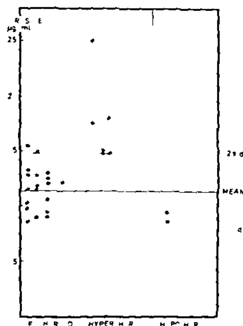


Fig 4 Results of fasting plasma tyrosine determinations in 39 euthyroid 36 hyperthyroid and 14 hypothyroid patients

patients with nodular goitre was almost the same being 11 4 ± 2 8 μg/ml These values are not significantly different from those of the control group

Plasma tyrosine levels during treatment with antithyroid drugs

Seven of the hyperthyroid patients were followed up with frequent determinations of plasma tyrosine for a period of 8—10 weeks The standard dose of anti thyroid drugs used was 200 mg propylthiouracil 3 times daily for 3 weeks followed by 300 mg daily One

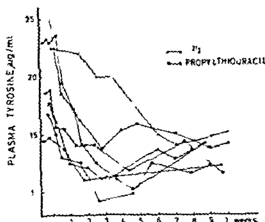


Fig 5 Results of fasting plasma tyrosine determinations during treatment with propylthiouracil and ^{131}I in 7 hyperthyroid patients

patient was followed up after treatment with 2 3 mC ^{131}I The plasma tyrosine levels are shown in fig 5 In all the patients treated with propylthiouracil the plasma tyrosine levels fell rapidly within 2—3 days and normal levels were reached in the course of a fortnight Simultaneous resin tri iodothyronine tests showed an almost parallel fall in the results The decrease in the plasma tyrosine level was much slower in the patient treated with ^{131}I and normal levels were not reached until about 8 weeks after the treatment In this patient free thyroxine was estimated by the method described by Korsgaard Christensen (6) and it was found that there was a decrease in free thyroxine parallel to that in the plasma tyrosine

Seventeen patients who had been treated with antithyroid drugs for more than 8 weeks were also studied All these patients were clinically euthyroid at the time of the investigation

The fasting plasma tyrosine levels were found to average 12 2 ± 3 2 µg/ml (mean ± 2 S D), a value which is not significantly different from the average for the control group

Tolerance test

Oral tolerance tests

Oral tolerance tests were carried out in 9 euthyroid 11 hyperthyroid and 3 hypothyroid patients In the majority of patients the peak values were obtained after 1 1/2 hours but during the period after administration the plasma tyrosine levels showed a very varied course The average fasting plasma tyrosine in the hyperthyroid patients was 19 0 µg/ml after loading a peak value of 39 0 ± 13 2 µg/ml (mean ± 2 S D) was obtained The average value for the fasting plasma tyrosine in the euthyroid subjects was 10 8 µg and after loading the peak values averaged 26 0 ± 11 6 µg/ml (mean ± 2 S D) In the individual patient there was no definite correlation between the fasting level and the highest level reached The average for the peak values in the hyperthyroid patients is significantly higher than the corresponding average in the euthyroid patients ($p < 0 001$) The average level of fasting plasma tyrosine in the hypothyroid patients was found to be 10 0 µg/ml and after loading the peak values in these patients averaged 27 7 ± 2 8 µg/ml (mean ± 2 S D) There is no significant difference between the peak levels in the hypothyroid and those in the euthyroid

TABLE I Fasting and peak plasma tyrosine values (in μg/ml) following oral loading with 0 05 g tyrosine/kg body weight in 9 euthyroid and 11 hyperthyroid subjects

Euthyroid		Hyperthyroid	
Fasting plasma tyrosine (μg/ml)	Peak plasma tyrosine (μg/ml)	Fasting plasma tyrosine (μg/ml)	Peak plasma tyrosine (μg/ml)
11 6	34 0	18 0	44 0
10 4	24 0	23 0	44 0
10 0	23 0	20 5	29 6
11 0	31 2	25 0	65 0
91 2	20 0	14 7	32 0
9 9	17 0	19 0	29 5
11 5	23 0	16 5	27 6
10 0	27 0	14 8	41 0
11 3	32 0	16 2	25 0
		23 3	44 0
		18 0	44 1

patients The results of the determinations after oral loading are shown in table I

Intravenous tolerance tests

Intravenous loading with 1 g tyrosine was carried out in 5 euthyroid 6 hyperthyroid and 4 hypothyroid patients The average fasting plasma tyrosine level in the hyperthyroid patients was 18 1 μg/ml that in the euthyroid was 11 1 μg/ml and in the hypothyroid 10 1 μg/ml Fig 6 shows the average plasma tyrosine levels reached at various times from 5 minutes to 4 1/2 hours after the tyrosine injection in each of the groups The average values for the hyperthyroid patients are significantly higher than those for the euthyroid patients in the period from 1/2 to 4 1/2 hours after the injection ($p < 0\ 001$) If however the individual fasting levels are subtracted from the results

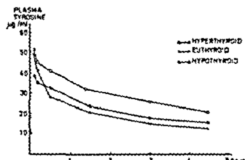


Fig 6 Mean plasma tyrosine values following injection of 1 g tyrosine intravenously in 5 euthyroid 6 hyperthyroid and 4 hypothyroid patients

obtained during the tolerance test it is found that the levels in the hyperthyroid patients are no longer significantly higher in the period 1/2 to 4 1/2 hours although they are still greater than the corresponding levels for the euthyroid patients ($p < 0\ 1$) There is no significant difference between the results of the intravenous tolerance tests in hypothyroid and euthyroid patients

The half life of the dose administered is almost the same in hyperthyroid and euthyroid patients The half life is somewhat longer in the hypothyroid patients but the difference is not significant

Discussion and conclusion

The fasting plasma tyrosine levels in euthyroid, hyperthyroid and hypothyroid patients are very close to those which have been reported hitherto The mean fasting plasma tyrosine levels which have been determined in the various investigations are shown in table II

The levels found in patients with atoxic goitre did not differ from those of the control group This would suggest that the goitre producing factor does not influence the regulation of the plasma tyrosine concentration

In patients treated with propyl thiouracil we found a marked fall in the

TABLE II Fasting plasma tyrosine

	Rivlin et al	Sos et al	Present studies
Euthyroid subjects	12 2±[a]4 9 (n=24)	12 5	11 3±3 6 (n=39)
Hyperthyroid subjects	18 5±9 5 (n=35)	20 0 (n=14)	18 8±6 2 (n=36)
Hypothyroid subjects	9 8±4 2 (n=12)		9 1±3 2 (n=14)

Mean values ±2 S D in μg/ml (n)=number of subjects
[a] Rivlin et al values in mean ± S E. is here converted into mean ± 2 S D

plasma tyrosine in the course of a few days (fig 5) The fact that the plasma tyrosine, the resin tri iodothyronine test, and the free plasma thyroxine returned to normal almost simultaneously might perhaps be thought to indicate that there is a relationship between the amount of free thyroxine and the plasma tyrosine, however, no relationship between the resin tri iodothyronine test and the plasma tyrosine level was found in untreated patients with hyperthyroidism

In 17 patients who had been treated with antithyroid drugs for at least eight weeks, and who were euthyroid at the time of investigation the plasma tyrosine levels were within normal limits This must indicate that propylthiouracil and other antithyroid drugs continue to have an inhibitory effect on the factors which influence the increase in plasma tyrosine in hyperthyroidism The plasma tyrosine level may therefore be of value in the assessment of the effect of treatment with antithyroid drugs

In an experiment in which we administered propylthiouracil in the doses used in the treatment of hyperthyroidism to euthyroid subjects we were unable to provoke a fall in plasma tyrosine It is possible that a short course of treatment with propylthiouracil could be used as an aid to diagnosis, as a marked fall in plasma tyrosine even in patients with levels within the normal range would suggest that the patient was thyrotoxic

As is apparent from fig 4 there is some overlap between the plasma tyrosine levels in the three groups It has already been mentioned that 14 % of the patients with hyperthyroidism and 71 % of those with hypothyroidism had levels which lay within the normal range It would therefore be an advantage if it were possible to obtain a clearer distinction between the groups by means of a tyrosine tolerance test Rivlin and Melmon (13) carried out an oral tolerance test using 50 mg tyrosine per kg body weight and obtained average peak values of approximately 100 μg/ml in hyperthyroid patients approximately 44 μg/ml in euthyroid subjects and approximately 30 μg ml in hypothyroid patients they also confirmed these observations in a later investigation (14) We have been unable to obtain similar levels, despite the fact that we have used a similar procedure in our investigations After loading the peak levels in 11 hyperthyroid patients averaged 39 0 ± 13 2 μg/ml (mean ± 2

S.D.), i.e. a far lower value. Perhaps the explanation of this is a difference in the technique used in the measurements of the higher concentrations of plasma tyrosine obtained in the samples after loading. Rivlin et al. (14), who used the same fluorometric method as that used in the present study, diluted the final aqueous phase of samples with high degrees of fluorescence with water (1 : 5 or 1 : 10). We have found that such 1 : 10 dilutions result in plasma tyrosine values which are about 145 % higher than the true concentration. By diluting with a solution having the same composition as the blind we obtained plasma tyrosine values which were in close approximation to those obtained by means of a colourimetric method. In the oral tolerance test we found that 45 % of the hyperthyroid patients had levels within the normal range, in contrast to the 14 % whose fasting plasma tyrosine levels lay within this range. On the basis of our limited material we must therefore conclude that the oral tolerance test cannot be considered to be of any diagnostic value.

In the intravenous tolerance test we found that initially there was an extremely rapid fall in the plasma tyrosine concentration. This has also been described in animal experiments (5). In these experiments it was not possible to discover any explanation of how the tyrosine is removed from the circulation so rapidly. There was no change in the half life in the blood of tyrosine administered to dogs after thyroidectomy.

The procedure which we have used for the intravenous tolerance test has led to significantly higher levels in the hyperthyroid patients. This difference can, however, be explained mainly on the basis of the raised fasting levels in these patients. Nonetheless, after the subtraction of the fasting values there is still a greater increase in the plasma tyrosine levels in the patients with hyperthyroidism. As the half life of the tyrosine is apparently the same in the two groups, this finding can perhaps be explained as the result of a reduction in the "tyrosine pool" in the hyperthyroid patients. More detailed investigation will be necessary before this can be established.

As there was no definite difference in the half life for the three groups of patients it would appear that the intravenous tolerance test, as used in the present investigation, cannot assist in a better differentiation between hyperthyroid, euthyroid and hypothyroid subjects.

A pilot study of the fasting plasma tyrosine levels in patients suffering from a wide variety of disorders has not revealed any deviation from the normal range. The investigation included patients with such disorders as diabetes mellitus, uraemia, hypertension, uncompensated heart failure, duodenal ulcer, cancer and phaeochromocytoma. This confirms the findings of other workers (3, 14). However, as yet no materials sufficiently large to permit of a more precise assessment of the plasma tyrosine levels in the different diseases have been published. In all events, raised plasma tyrosine levels have been found in patients with parenchymatous liver damage and the use of determination of plasma tyrosine

as a test of thyroid function is thus impracticable in patients with such disease

The cause of the changes in plasma tyrosine concentration which have been found in hyperthyroidism and hypothyroidism has not been elucidated

An obvious explanation of the changes in plasma tyrosine would be that thyroxine has an inhibitory effect on the tyrosine transaminase in liver tissue as this enzyme is essential for the metabolism of tyrosine Investigations of rats treated with thyroxine have revealed that there is an increased activity of the tyrosine transaminase in the liver, without any change in the phenylalanine hydroxylase activity (12) In contradistinction to this Litwach (8) found in in vitro experiments that thyroxine inhibits the tyrosine transaminase in liver tissue Parenchymatous liver disorders lead to a marked increase in plasma tyrosine (1), but the results mentioned above would suggest that the liver enzyme systems are not the only factors of importance in the regulation of the plasma tyrosine level In addition animal experiments have also revealed that the administration of insulin and glucose to hepatectomized rats can counteract any increase in the plasma amino acids (3) Paper electrophoretic studies of the free amino acids in liver muscle and renal tissue from rats treated with thyroxine and thyroidectomized rats revealed no significant deviation from the normal (3) It is conceivable that the increased concentration of tyrosine in hyperthyroidism is an expression of a general increase in amino acid metabolism However Melmon et al (9) have determined the concentrations of 17 amino acids in the plasma of eight patients with hyperthyroidism and found that only the levels of tyrosine and glutamine were raised

The results of the oral tolerance test would suggest that the raised plasma tyrosine in thyrotoxicosis is not due to increased absorption from the alimentary tract Rivlin and Melmon (13) have investigated the turnover of ^{14}C-labelled tyrosine in rats treated with thyroxine, and found a reduction in the tyrosine pool' and increased tyrosine metabolism

We consider it reasonable to conclude that the estimation of fasting plasma tyrosine is of value as a supplement to the other tests of thyroid function The method is straightforward and has the advantage that, like the resin tri iodothyronine test the results are not affected by any possible intake of iodine before the investigation The method is of little value in the diagnosis of myxoedema or in the investigation of patients with parenchymatous liver disease The determination of the fasting plasma tyrosine will be of special value in the diagnosis of thyrotoxicosis in iodine-contaminated patients and also in patients in whom the results of the resin-tri iodothyronine test are erroneously high, for example those suffering from cancer uraemia and other disorders with disturbances of protein metabolism

Summary

In previous investigations the level of the amino acid tyrosine in the plasma has been found to be increased in patients

with thyrotoxicosis, and decreased in those with myxoedema.

An investigation has been carried out with the aim of assessing the value of the determination of plasma tyrosine in the diagnosis of thyroid diseases. Thirty six hyperthyroid, 14 hypothyroid, and 39 euthyroid patients were studied. The fasting plasma tyrosine was 18.8 ± 6.2 μg/ml (mean ± 2 S.D.) in the hyperthyroid, 9.1 ± 3.2 μg/ml in the hypothyroid, and 11.3 ± 3.6 μg/ml in the euthyroid patients. The average values for the three groups differed significantly one from another ($p < 0.001$). Eighteen patients with atoxic goitre had levels which lay within normal limits. During treatment with antithyroid drugs the plasma tyrosine fell rapidly to normal levels and there were also falls in the resin tri-iodothyronine test and in free thyronine. In 17 previously hyperthyroid patients who had been treated for long periods with antithyroid drugs the plasma tyrosine levels were normal.

An attempt was made to design oral and intravenous tolerance tests for the diagnosis of thyroid disorders, but these were found to be of no greater value that the determination of the fasting plasma tyrosine.

We consider it justified to conclude that the determination of fasting plasma tyrosine is valuable as a supplement to the other tests of thyroid function. The method is straightforward and shares the advantage with the resin tri-iodothyronine test of being unaffected by any possible administration of iodine before the investigation. The test is of no value in myxoedema, and the results are affected in the presence of parenchymatous liver damage.

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The Effect of Parathyroid Hormone on the Tubular Reabsorption of Glucose

By

Bent Halver

In 1908 Hirsch (4) demonstrated glycosuria in dogs following extirpation of the parathyroid glands Subsequent workers found that extirpation of 3 or more parathyroid glands in dogs was followed by a decreased glucose tolerance The glycosuria was considered as secondary to increased blood glucose levels but recently other investigators have failed to demonstrate any influence of parathyroid extract (PTE) on the blood glucose levels (6 12) No investigation seems to have been made to decide whether parathyroid hormone (PTH) exerts any influence on the renal threshold of glucose — the question dealt with in this report

Material and methods

The material consists of 4 patients Two patients (No 1 and No 2) had primary hyperparathyroidism verified by the finding of adenomas during operation Two patients (No 3 and No 4) had post surgical hypoparathyroidism substantiated by the finding of subnormal values of plasma calcium for several years after strumectomy

Submitted for publication September 16 1965

The maximal rate of tubular reabsorption of glucose (TmG) was calculated from determinations of the glomerular filtration rate (GFR) the plasma glucose (PG) and urine glucose concentrations (UG) and the diuresis (V)

TmG (mg/min) = PG (mg/100 ml) GFR (ml/min) — UG (mg/100 ml) V (ml/min)

Estimation of GFR was based on determination of inulin clearances except in one patient (No 1) where the endogenous creatinine clearance was used Adequate hydration was achieved by the administration of 800—1 000 ml of water by mouth to the fasting patients during one hour In patient No 1 the glucose was administered by infusion of 0 74 g per minute for 50 minutes 1 10 g per minute for the next 50 minutes and 1 80 g per minute for the following 30 minutes In patient No 2 the glucose was administered by constant infusion of 0 5 g per minute for 60 minutes 0 8 g per minute for other 60 minutes and 1 7 g per minute for the following 60 minutes The inulin was given as a priming infusion of 0 170 g per minute for 30 minutes before the glucose was given followed by sustained infusion of 0 050 g per minute Twenty per cent glucose solution and 10 per cent inulin solution were used The stated values of TmG and TmG/GFR

TABLE II Estimation of the mean values of TmG/GFR n 3 subjects w thou t diseases of the para thyroid glands or of the carbohydrate metabolism

	Period	M n	GFR (ml/m n)	TmG (mg/m n)	TmC/CFR
Subject no 5	1	15	97	223	? 30
	2	15	92	220	2 39
	3	15	83	185	2 23
	4	15	95	184	1 94
			92	203	2 22±0 19
Subject no 6	1	15	122	303	2 48
	2	15	130	305	2 35
	3	15	128	308	2 41
	4	15	127	294	2 31
			127	303	2 39±0 07
Subject no 7	1	15	101	206	2 04
	2	15	116	256	? 21
	3	15	148	336	2 27
	4	15	127	263	2 07
			123	265	2 15±0 11

TmG GFR 2 25 0 16 (SD)

TABLE III Laboratory alues llustrat ng the parathyro d func on of pat ent no 2 Determ nations of the calcium fractions ha e been performed by S Hahnemann M D Frederiksberg Hosp tal A mod f cat on of Rose s original method was used (9)

Patient no 2	Total calc n (g 100 n l) 9 4 10 (	D ff s ble calc m (mg 100 n l) (7 7 5	Ion zed cal m (g/100 ml) 1 0 6 6	Alkal ne pho ph a ses (k A U /100 ml) 3 (11	Ac d pho ph a ases K A U /100 ml) 3 7	Phosphate (g/100 n l) 2 5 4 (	C n g 100 1 7 2 7
2 days before op	16 0	12 5	10 8	129	13 4	2 7	4 5
11 days af er op	7 8	5 8	5 1	155	0 7	2 2	1 8
8 weeks after op	8 8	6 8	5 7	64	4 0	3 2	2 9

those quoted possibly because in this study glucose was determined in venous plasma instead of arterial plasma An arteriovenous glucose deficit of 10 per cent will raise these values of TmG and TmG GFR by 25 3 ± 9 6 per cent

The parathyroid function of patient No 2 has been estimated from determi

nations of the plasma calcium fractions the alkaline and acid phosphatases and plasma citric acid (table III) When entering the hospital the patient had severe bone disturbances which explain the high values of alkaline phosphatases At the operation a very large adenoma was removed, which explains why 11 days after the operation the patient still was in a state of hypoparathyroidism as indicated by the low values of the acid phosphatases plasma citric acid and plasma calcium and by paresthesias of the hands Eight weeks after the operation the supranormal values of the acid phosphatases and of the plasma citric acid indicated that the patient now had entered a state of secondary hyperparathyroidism The low plasma values of ionized calcium caused by a pronounced bone avidity for calcium most likely have stimulated the parathyroid glands to this secondary hyperfunction

Strong evidence suggests that both phosphate and glucose are reabsorbed in the proximal tubules and in all probability under a competitive inhibition as indicated by the findings of a parallelism between blood glucose level and phosphate clearances (2 5 7, 11) Possibly the influence of PTH on the reabsorption of glucose shown in this study is due to the inhibition of the tubular reabsorption of phosphate which might make a correspondingly larger part of the common transport apparatus available for the tubular transport of glucose

Investigations are in progress to decide whether these findings might be valuable for the differential diagnosis of hypercalcemias due to parthyroid and non parathyroid diseases

Summary

The following observations indicate the existence of an effect of the parathyroid hormone on the maximal rate of reabsorption capacity for glucose (TmG) and TmG/GFR (TmG related to the glomerular filtration rate)

1 In two cases of primary hyperparathyroidism TmG and TmG/GFR was found to be elevated

2 During the period of transitory hypoparathryroidism following parathyroidectomy in one of these cases a pronounced decrease of TmG and TmG GFR was found

3 Eight weeks after the parathyroidectomy the same patient seems to have entered a phase of secondary hyperparathyroidism At this time the patient revealed an elevation of TmG and TmG GFR these reaching supranormal values

4 Subnormal values of TmG and TmG/GFR were demonstrated in two cases of post-surgical hypoparathyroidism

5 Following intramuscular administration of parathyroid extract the same two patients revealed a statistically significant increase of TmG and TmG/GFR

The mechanism by which the parathyroid hormone may influence TmG is discussed

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From the Department of Medicine IV Sodersjukhuset Stockholm Sweden

The Serum Creatine Phosphokinase Activity and the Achilles Reflex in Hyperthyroidism and Hypothyroidism

By

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It is a well established fact that both hyperthyroidism and hypothyroidism are often associated with muscular symptoms

In thyrotoxicosis muscular weakness is an important symptom as already emphasized by Basedow (2) and Graves (7) in their original descriptions A number of cases of thyrotoxicosis with marked paresis and atrophy have been reported under the diagnosis of chronic thyrotoxic myopathy Clinical histological and electromyographical studies have shown that the muscular weakness present in almost every case of thyrotoxicosis is in fact caused by a true myopathy (10)

In hypothyroidism muscular pain stiffness and cramps are common symptoms The muscle reflexes show prolonged contraction and relaxation Pseudomyotonia (12 21) is a comparatively frequent finding and can most easily be recorded in the Achilles reflex (3 14) Åstrom et al (1) have demonstrated that the generalized reduction in muscular strength often occurring in hypothyroidism is to be ascribed to a true myopathy

In order to obtain further information about the role of the muscles in thyroid disorders we determined the creatine phosphokinase (CPK) activity in serum and recorded the Achilles reflex by the Kinemometer test in both thyrotoxicosis and myxoedema The patients were investigated before and during treatment The present paper comprises a report and discussion of the results

Methods

Protein bound iodine (PBI) in serum was determined according to Skanse and Hedenskog (20) Normal value 4—8 μg/100 ml

Radioactive iodine The uptake in the thyroid gland and the urinary output were measured after 24 hours Uptake after 24 hours normal value 15—45 % of the dose administered borderline value to thyrotoxicosis 45—60 % pathologically raised value > 60 % (22 In a few cases this test was complemented by measurement of the uptake in the thyroid after 2 4 7 and 30 hours and by determination of the *biological half life of* ^{131}I Normal value > 24 days (22

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TABLE I. Case material

Case no	Diagnosis	Sex	Age (yrs)	BMR	Serum cholesterol (mg/100 ml)	PBI (µg/100 ml)	¹³¹I Uptake	¹³¹I Excretion
							(%/24 hrs)	
1	Thyrotoxicosis	♀	55	+24	152	11.6	68	7
2	Thyrotoxicosis	♀	63	+41	216	8.7	69	11
3	Thyrotoxicosis	♀	63	+43	144	18.4	—	—
4	Thyrotoxicosis	♀	58	+57	148	17.4	88	3
5	Thyrotoxicosis	♀	79	+46	130	[1]>20.0	64	16
6	Thyrotoxicosis	♀	77	+43	208	12.0	68	13
7	Thyrotoxicosis	♀	55	+44	204	12.3	[1]52	17
8	Thyrotoxicosis	♀	60	+87	120	17.3	[1]45	12
9	Thyrotoxicosis	♀	57	+28	192	13.8	[1]59	22
10	Thyrotoxicosis	♀	63	+43	—	10.1	80	17
11	Thyrotoxicosis	♂	70	+56	192	8.5	57	14
12	Primary myxoedema	♀	68	−18	240	2.0	10	20
13	Primary myxoedema	♀	45	−29	700	2.9	12	26
14	Primary myxoedema	♀	69	−16	330	2.0	8	50
15	Primary myxoedema	♀	51	−18	520	2.0	—	—
16	Myxoedema after ¹³¹I treatment	♀	69	− 8	—	1.8	8	79
17	Myxoedema after ¹³¹I treatment	♀	65	+ 6	236	2.8	22	53
18	Myxoedema after op. atoxic goitre	♀	64	−19	292	2.8	4	74

[1] Cf. text

Creatine phosphokinase (CPK) activity in serum was determined by the method of Hughes (13). The controls consisted of 28 *ambulant hospitalized patients (18 men and* 10 women) with no signs of muscle or heart disease. CPK range in the controls 0.3—4.1 units (1 unit = 1 µmol creatine/ml serum/hour at 37° C, ref. 16).

Achilles reflex. This was recorded with the Kinemometer method described by Lawson (14), in which tracings are obtained of the contraction and relaxation phases of the Achilles reflex. The contraction phase (S-D interval) normally amounts to 200—280 msec (4).

Material

The material consisted of 18 patients: 1 man and 10 women with thyrotoxicosis and 7 *women with myxoedema (table I).*

In the *hyperthyroid group*, 4 patients (cases 4 and 7—9) had moderate or severe paresis of proximal muscle groups before treatment. These patients presented the clinical features of chronic thyrotoxic myopathy. In the other 7 cases, clinical examination disclosed no definite weakness of proximal muscles.

In the *hypothyroid group*, 4 patients (cases 12—15) had primary myxoedema. One of them (case 13) also had a nephrotic syndrome. Pernicious anaemia was present in case 12.

In cases 16 and 17 myxoedema had arisen after treatment of thyrotoxicosis with radioactive iodine In case 18 hypothyroidism had developed after surgical treatment of an atoxic nodal goitre

All the patients with myxoedema had muscular pain and stiffness Case 18 also had fairly severe attacks of cramp in the calves No proximal muscular weakness could be demonstrated in any of the 7 cases Two patients (cases 12 and 14) were however, in such poor general condition that it was impossible to judge whether any such weakness was actually present In 6 patients the Achilles reflex showed a prolonged relaxation time at the clinical examination In case 12 both patellar and Achilles reflexes were absent

Diagnosis The diagnostic criteria used were in addition to the clinical examination determinations of the basal metabolism rate (BMR) cholesterol and PBI in serum and radioactive iodine tests The results of these determinations are listed in table I

In *case 5* an extremely high PBI value was recorded ($> 20\ \mu g/100$ ml) The patient was found to have taken an iodine-containing preparation a few weeks before the test The results of other investigations (BMR serum cholesterol radioactive iodine) were indicative of thyrotoxicosis Since the symptoms of thyrotoxicosis completely disappeared on treatment with methylthiouracil the diagnosis must be regarded as established

In 3 patients (cases 7 8 and 9) the radioactive iodine test showed borderline values These analyses were therefore complemented by determination of the uptake after 2 4 7 and 30 hours as well as determination of the biological half life of ^{131}I *Case 7* had an uptake in the thyroid gland of 56 and 56 % after 4 and 7 hours respectively The 24 hour urinary output was 7 % Biological half life of ^{131}I 8 4 days *Case 8* had an uptake of 68 and 66 % after 2 and 4 hours respectively Urinary output 7 % Biological half life of ^{131}I 6 5 days *Case 9* had an uptake of 29 44 59 and 67 5 % after 2 4 24 and 30 hours respectively Urinary output 28 % Biological half life of ^{131}I 15 7 days

Thus in all three cases the complementary determinations showed a pathologically high uptake of radioactive iodine in the thyroid gland The biological half life of ^{131}I was low Moreover the urinary output of creatine was pathological in every case The creatinuria ceased after treatment with methylthiouracil Consequently the diagnosis of thyrotoxicosis must be considered as established in these cases as well

Therapy In the thyrotoxicosis group the initial dose of methylthiouracil was 0 4—0 6 g daily The dose was successively reduced with clinical improvement When the patient was euthyroid a therapeutic dose of radioactive iodine was given

The hypothyroid patients were treated with a synthetic thyroid preparation L-thyroxine sodium (Levaxin ®) The initial dose was low and ranged from 0 025—0 05 mg daily It was increased to an optimal effect after which the maintenance dose ranged from 0 15—0 3 mg daily

Results

Hyperthyroidism

Creatine phosphokinase (CPK) in serum (table II) This was determined in 10 cases Before treatment the CPK was normal in every case (range 0 4—3 5 units)

On treatment with methylthiouracil a pathological rise in serum CPK occurred in 3 patients (cases 1 4 and 7) In case 1 the CPK value was 3 2 units before treatment and rose to 16 7 units In case 4, the corresponding rise was from 1 4 to 7 2 units and in case 7 from 0 8 to 13 9 units No clinical signs of myxoedema appeared in any of these three patients

Kinemometer test (table III) Ten patients were tested before treatment In

TABLE II Creatine phosphokinase (CPK) in serum in hyperthyroidism

Case no	Serum CPK (units) Before treatment	After treatment 1	2	3	4	6	8	12	16	20 weeks
1	3.2	—	1.6	2.5	1.9	8.6	8.6	9.7	16.7	—
2	0.4	1.2	—	—	0.8	0.9	1.1	0.6	0.5	—
3	3.5	—	—	3.5	—	—	—	2.3	—	—
4	1.4	—	—	—	—	—	—	7.2	3.1	2.0
5	1.5	0.5	0.5	—	1.0	0.7	0.9	1.2	1.5	—
6	0.8	0.9	0.7	—	0.9	—	—	—	—	—
8	0.7	1.0	—	—	0.4	—	2.5		—	—
9	1.2	0.8	0.5	—	0.6	0.7	0.7	—	—	—
11	0.6	—	0.9	—	—	—	—	—	—	—

Case no	Before treatment	1	2	3	4	6	8	9	10 weeks
7	0.8	0.8	1.5	3.6	—	11.3	13.9	6.9	2.6

TABLE III Kinemometer test in hyperthyroidism

Case no	S — D interval (msec) Before treatment	After treatment 1	2	3	4	6	8	12	16	20 weeks
1	180	183	198	—	224	271	240	229	268	—
2	184	196	193	202	206	207	221	—	—	—
3	215	209	—	—	—		—	—	—	
4	—	—	—		—	—	—		257	
5	183	202	200	213	199	200	238	231	—	
6	191	—	—				—	—	—	
8	183	—	209	233			204	233	—	
9	185	203	187	—	208	236		—	—	
10	191	191	191	—	214		—	—	—	
11	190	—	218	—	213	—	—		—	

Case no	Before treatment	1	2	3	4	6	8	9	10 weeks
7	206	210	217	210	224	245	299	278	238

TABLE IV Creatine phosphokinase (CPK) in serum in hypothyroidism

Case no	Serum CPK (units) Before treatment	After treatment								
		1	2	3	4	6	8	12	16	20 weeks
12	13 5	—	1 8	—	2 8	3 2	1 8	0 7	—	—
13	31 6	19 7	8 3	11 9	—	10 5	4 9	—	1 5	—
14	42 0	7 4	—	5 4	6 0	4 4	3 6	3 0	—	—
15	13 0	—	—	—	—	—	—	—	—	—
17	12 0	4 2	2 1	1 3	1 6	—	—	—	—	—
18	15 4	10 2	19 4	—	9 5	6 1	4 3	2 0	—	1 2

TABLE V Kinemometer test in hypothyroidism

Case no	S-D interval (msec) Before treatment	After treatment								
		1	2	3	4	6	8	12	16	20 weeks
13	400	—	400	380	—	—	348	333	269	248
14	315	275	—	—	280	274	265	—	239	—
15	300	—	—	—	—	—	—	—	—	—
16	295	267	247	242	—	—	256	265	230	—
17	300	—	—	—	—	—	—	—	—	—
18	285	—	—	—	247	271	229	231	227	231

8 cases a pathologically short S D interval was recorded i e < 200 msec (range 180—191 msec) In cases 3 and 7 low normal values were recorded (215 and 206 msec respectively)

Methylthiouracil medication resulted in every case observed in normalization of the S D interval In cases 5 and 9 this occurred within 1 week Cases 8 and 11 were not tested until after 2 weeks treatment when normal S D values were noted In case 2 return to normal took place after 3 weeks and in cases 1 and 10 after 4 weeks, the latter patients were not however tested after 3 weeks

Hypothyroidism

Creatine phosphokinase (CPK) in serum (table IV) Determinations were made in 6 cases All had a pathologically raised

TABLE II Creatine phosphokinase (CPK) in serum in hyperthyroidism

Case no	Serum CPK (units) Before treatment	After treatment								
		1	2	3	4	6	8	12	16	20 weeks
1	3 2	—	1 6	2 5	1 9	8 6	8 6	9 7	16 7	—
2	0 4	1 2	—	—	0 8	0 9	1 1	0 6	0 5	—
3	3 5	—	—	3 5	—	—	—	2 3	—	—
4	1 4	—	—	—	—	—	—	7 2	3 1	2 0
5	1 5	0 5	0 5	—	1 0	0 7	0 9	1 2	1 5	—
6	0 8	0 9	0 7	—	0 9	—	—	—	—	—
8	0 7	1 0	—	—	0 4	—	2 5		—	—
9	1 2	0 8	0 5	—	0 6	0 7	0 7	—	—	—
11	0 6	—	0 9	—	—	—	—	—	—	—
		1	2	3	4	6	8	9	10 weeks	
7	0 8	0 8	1 5	3 6	—	11 3	13 9	6 9	2 6	

TABLE III Kinemometer test in hyperthyroidism

Case no	S — D interval (msec) Before treatment	After treatment								
		1	2	3	4	6	8	12	16	20 weeks
1	180	183	198	—	224	271	240	229	268	—
2	184	196	193	202	206	207	221	—	—	—
3	215	209	—	—	—	—	—	—	—	—
4	—		—	—	—	—	—	—	257	—
5	183	202	200	213	199	200	238	231	—	—
6	191	—	—			—	—	—		—
8	183	—	209	233		—	204	233	—	—
9	185	205	187	—	208	236	—	—	—	—
10	191	191	191	—	214	—	—	—	—	—
11	190		218	—	213	—	—	—	—	—
		1	2	3	4	6	8	9	10 weeks	
7	206	210	217	210	224	245	299	278	238	

found a shortening of the contraction phase (S D interval) when testing the Achilles reflex in patients with thyrotoxicosis In nearly all our cases of thyrotoxicosis pathologically short S D intervals were recorded, i e , < 200 msec However, since normal values were present in two cases, a normal speed of reflex does not rule out the existence of hyperthyroidism Methylthiouracil therapy produced a successive normalization of the S D interval

A raised CPK activity in serum in hypothyroidism was reported by Graig and Ross (5) No correlation was present between the PBI level and the uptake of radioactive iodine respectively and the raised enzyme level Similar results have subsequently been reported by other workers (6 8 18) Hess et al (11) on the other hand found a raised CPK level in only 1 of 5 hypothyroid patients

Raised values for CPK in serum and prolongation of the S D interval were recorded in all our hypothyroid patients in whom these determinations were made After institution of L thyroxine medication both values returned to normal

A remarkable feature is that the CPK activity in serum is normal in thyrotoxicosis but pathologically raised in myxoedema despite the clinical muscle symptoms in the form of paresis and atrophy being far more pronounced in the former disorder than in the latter This is also illustrated by the present material

Slight histological lesions of the muscles indicative of recurrent muscle damage with subsequent healing processes have been demonstrated in myxoedema (1) Such histological changes are lacking to a great extent in thyrotoxicosis (10) Consequently it is possible that the raised CPK level in myxoedema is caused by slight, repeated muscle damage

In three of our patients with thyrotoxicosis methylthiouracil treatment was associated with a definitely pathological rise in serum CPK In cases 1 and 7, in whom repeated Kinemometer tests were made this rise was recorded after 6 weeks treatment On this occasion, normal S D intervals were recorded In case 1 no pathological lengthening of the S D interval occurred despite a rise in CPK to 16 7 units after 16 weeks medication In case 7, the CPK value after 6 weeks therapy was 11 3 units whereas the S D interval was normal (245 msec) After 8 weeks treatment the CPK was 13 9 units and the S D interval had increased to 299 msec Methylthiouracil medication was then discontinued One week later the Kinemometer value had returned to normal, whereas the CPK activity was still raised

Two hypothyroid patients (cases 14 and 18) showed a normal S D interval several weeks before the serum CPK had become normal In another hypothyroid patient (case 13) an unusually high S D value was recorded i e 400 msec Treatment produced a slower decrease in the S D interval than in the other cases thus it became normal at the same time as the CPK level This patient also had a nephrotic syndrome which probably contributed to the slow normalization of the S D interval Harrel and Daniel (9) did in fact observe a pro

longation of the Achilles reflex time in a patient with nephrosis

In view of these observations, it is possible that a pathologically raised serum CPK value during treatment with methylthiouracil may be an early sign of hypothyroidism

It is also probable that in treatment of hypothyroidism with thyroid preparations, a raised serum CPK activity persists for a longer time than does a pathological prolongation of the S D interval

Furthermore, the results of the present investigation indicate that, in thyroid disorders, the CPK activity in serum is a more sensitive indicator of hypothyroidism than is the duration of the S D interval of the Achilles reflex in the Kinemometer test

Summary

In a series of 11 hyperthyroid and 7 hypothyroid patients, determinations have been made of the creatine phosphokinase (CPK) activity in serum, and/or the duration of the contraction phase (S D interval) of the Achilles reflex in the Kinemometer test

In the hyperthyroid group, normal CPK values were recorded, whereas the Achilles reflex was pathologically shortened in 8 cases and normal in 2 In all cases tested, methylthiouracil therapy resulted in normalization of the Achilles reflex

In every case in the hypothyroid group, pathologically raised CPK values were recorded, as well as prolongation of the Achilles reflex On treatment with L-thyroxine in all cases tested both the CPK and the Achilles reflex returned to normal

Acknowledgement

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Allergic Phenomena Precipitated by Muscular Effort

By

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In a previous paper attention was called to the importance of effort for eliciting various allergic syndromes (1) the pioneer observation to the best of my knowledge being that made by Joltrain (4) Papers with bearing on similar topics have been published at a later date in Sweden (2 3) as well as abroad (5) The present report intends to add some few further observations to the topic

Case reports

Case 1 L A man born Aug 5 1931 In 1939 epidemic hepatitis In 1948 respiratory infection pains in the knee joints and 3 weeks later jaundice Observed in the clinic Nov 16th 1948—June 6th 1949 Conclusive signs of hepatitis at the bedside as well as on the laboratory records When in Feb 1949 he was allowed to be up and about there were to be observed nearly palmsized strictly limited red areas on the fronts of the calves down to the dorsum pedis These eruptions were very similar to urticaria and were easily made to disappear if the patient was allowed to recline in a supine position and with elevation of the legs On the other hand they could be provoked either by standing for 10—15 minutes or by walking around We tried to make him ride a test bicycle the areas did not appear on this test but if he stepped down from the cycle-contraption and walked slowly around they appeared again This phenomenon was transient already after one month it was less pronounced than when first noticed In May 1951 he was readmitted to the clinic presenting a full blown picture of liver cirrhosis with ascites, caput Medusae (veins going from the umbilicus) spider naevi on his hands acne of the fronthead and horizontal upper limit of the pubic hair On this occasion when he was fairly well from his own point of view the skin phenomenon already mentioned was not to be elicited The course in the clinic was uneventful but some time afterwards he died the necropsy confirming the diagnosis

In this case of chronic hepatitis urticaria like plaques were to be provoked in the skin of the legs by walking around

Case 2 Lady W 46 years of age Some 8 years before she had severe hepatitis She was now in a fairly good condition and the laboratory tests of the liver function were fairly normal The only symptom of which she complained was that she was apt to get easily exhausted by muscular effort She had observed a peculiar phenomenon which to herself was heralding and accompanying her fatigue she felt a peculiar taste-sensation reminding her of pepper

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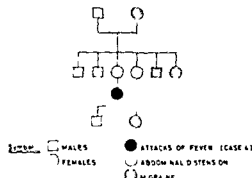


Fig 1 Diagram on the pedigree of case 4 Black symbol case 4 Suppled symbol attacks of fever and swelling of the abdomen and the hands on muscular effort Striated symbols migraine

Later I have heard the same symptom described by other patients who have had severe hepatitis although it is by no means constant and as a rule is only elicited by questioning In this case muscular fatigue was recorded by the patient as a peculiar taste sensation

Case 3 C O boy born June 29th 1943 the son of a colleague I was consulted when the boy was 16 years of age Since the age of 14 he had had attacks of Quincke-oedema affecting the eye lids making him unable to open his eyes These attacks were to be elicited by muscular effort only for instance when running through the woods when swimming when playing hand ball in a gymnasium which was new and very clean Each attack developed during the course of 10—15 minutes started to recede after 6—8 hours disappeared completely in 48 hours This boy was advised to avoid muscular effort and to give up gymnastics whereupon he had no further oedema Now in 1965 he has not had any attacks for 2 years and is doing his military service without any inconvenience

In this case a severe Quincke-oedema of the eyelids was precipitated by muscular effort

Case 4 I S Woman born Nov 30th 1917 Repeatedly observed by myself in the department of medicine in Lund during the years 1943 1944 and 1946 and followed up in 1961 and 1963 Seen by myself July 1965

Family history Her grandmother had migraine She had 6 children 3 sons (one of these uncles got severe migraine) and 3 daughters The youngest daughter (= aunt of the patient) had severe migraine The mother of the patient who is now well and alive at 80 had since the age of 40—50 had repeated attacks of fever for 3—4 days at a time These attacks started as a rule with a distension of the abdomen and with an increased size of her hands which became painlessly swollen Gradually then the temperature rose to 39 C° and then subsided so that the whole attack was over after 4 days Such an attack may be precipitated by ironing

The patient herself has 2 children One boy born May 1948 is healthy and does not complain of any headaches The girl who is 5 years his junior has frequent headaches starting already when she was 2 years old This headache is particularly pronounced if she has been doing horse riding dancing or gymnastics She is very ambitious and always carries her exercise to the limit of her possibilities

Past history As a child measles scarlet fever, German measles and chickenpox During her school years entirely healthy although slight abdominal disturbances during the years 1935—1940 (constipation pains in the epigastrium elicited one hour after consumption of fried potatoes or after exposure to cold)

Present history In 1937 she had during a visit to Poland on two occasions attacks of high fever lasting one day only In 1938 the same fever for 4 days Since the Christmas of 1942 she has had repeated attacks of fever elicited by muscular effort and accompanied by chills as well as pains in the bones of her calves occasionally also in her fore arms and her head (as a cap on her head) When the fever is on the increase she always wants to extend her arms and her legs as if she should want to yawn and she wants to gasp

although she feels unable to do so Repeated examinations in the clinic failed to reveal anything abnormal with regard to basal metabolic rate haematology (also osmotic resistance of red cells) sedimentation rate cholecystography and blood cultures She maintains that her sclerae are apt to get a yellowish tinge after each attack of fever although we have never been able to confirm that In 1958 cholecystectomy (before that pains in the liver region and in the lymph nodes of groins axillae and neck at each period of fever) The attacks however did not stop Occasionally, as in 1945 the fever was accompanied by a severe hunger When the fever is increasing she is apt to get an attack of urina spastica, voiding large amounts of urine colour like water She cannot tolerate any muscular effort When for instance she is ironing she becomes increasingly cool has to put on a cardigan and afterwards if she goes on with the ironing she gets a temperature of 40 C° The same is the case when she runs

Pregnancies Her first delivery was May 9th 1948 For the first 3 months of this pregnancy she had some attacks of fever as well only during the last 6 months she felt unusually able and strong When menstruation started again 2 1/2 months after delivery the attacks of fever returned She nursed her baby herself and produced very large quantities of milk The baby was fed 5 times a day and each nursing was accompanied by a fever of 40 C so that she could have 5 such attacks in 24 hours Male hormones were administered stopping lactation immediately as well as the subsequent attacks of fever However her attacks returned until her second pregnancy (in 1953) when she was absolutely healthy for all 9 months (the last 3 months of this pregnancy I was able to perform more physical work than I ever had been able to do) The nursing was uneventful since if she got tired at a meal of the baby she omitted it However 6 days before each menstruation she got an attack of fever and the next attack came when menstruation was finished occasionally however also in the middle between two menstruations She was attended by a physician in the town where she had settled, and moderate amounts of gammaglobulin were used with some effect to alleviate her symptoms

"There is something the matter with my muscles I always feel discomfort and fatigue about them when an attack is about to appear There has never been any brown or red colour of her urine in connection with an attack the only urinary finding has been the water like appearance in connection with the urina spastica at the onset of an attack

The syndrome of this woman with fever as its outstanding feature is not quite clear as to its origin No discolouration of the urine was observed at any time otherwise a paroxysmal myoglobinuria might have been suspected (6) It should be readily admitted that no analysis along this line had been carried out (spectrophotometrically or electrophoretically) The one outstanding feature in this case was the reaction after muscular effort and it seems reasonable to assume a response from the body towards some intermediate compound in this connection The second remarkable feature about this patient was the familial occurrence of migraine which in the case of her daughter was obviously elicited by strenuous muscular activity

Comments and conclusions

The common features in these rather unrelated instances was the presence of a muscular effort, inducing in one case urticaria in another a peculiar taste-sensation, in the third case a Quincke-oedema and in the fourth case attacks of fever

In quite another disease muscular effort is well known as a precipitating factor In patients with gout the attacks tend to accumulate and to grow more severe if the patient is using his legs

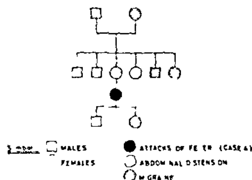


Fig 1 Diagram on the pedigree of case 4 Black symbol case 4 Stippled symbol attacks of fever and swelling of the abdomen and the hands on muscular effort Striated symbols migraine

Later I have heard the same symptom described by other patients who have had severe hepatitis although it is by no means constant and as a rule is only elicited by questioning In this case muscular fatigue was recorded by the patient as a peculiar taste sensation

Case 3 C O boy born June 29th 1943 the son of a colleague I was consulted when the boy was 16 years of age Since the age of 14 he had had attacks of Quincke-oedema affecting the eye lids making him unable to open his eyes These attacks were to be elicited by muscular effort only for instance when running through the woods when swimming when playing hand ball in a gymnasium which was new and very clean Each attack developed during the course of 10—15 minutes, started to recede after 6—8 hours disappeared completely in 48 hours This boy was advised to avoid muscular effort and to give up gymnastics whereupon he had no further oedema Now in 1965 he has not had any attacks for 2 years and is doing his military service without any inconvenience

In this case a severe Quincke-oedema of the eyelids was precipitated by muscular effort

Case 4 I S Woman born Nov 30th 1917 Repeatedly observed by myself in the department of medicine in Lund during the years 1943 1944 and 1946 and followed up in 1961 and 1963 Seen by myself July 1965

Family history Her grandmother had migraine She had 6 children 3 sons (one of these uncles got severe migraine) and 3 daughters The youngest daughter (= aunt of the patient) had severe migraine The mother of the patient who is now well and alive at 80 had since the age of 40—50 had repeated attacks of fever for 3—4 days at a time These attacks started as a rule with a distension of the abdomen and with an increased size of her hands which became painlessly swollen Gradually then the temperature rose to 39 C and then subsided, so that the whole attack was over after 4 days Such an attack may be precipitated by ironing

The patient herself has 2 children One boy born May 1948 is healthy and does not complain of any headaches The girl, who is 5 years his junior, has frequent headaches starting already when she was 2 years old This headache is particularly pronounced if she has been doing horse riding dancing or gymnastics She is very ambitious and always carries her exercise to the limit of her possibilities

Past history As a child measles scarlet fever, German measles and chickenpox During her school years entirely healthy, although slight abdominal disturbances during the years 1935—1940 (constipation pains in the epigastrium elicited one hour after consumption of fried potatoes or after exposure to cold)

Present history In 1937 she had during a visit to Poland on two occasions attacks of high fever lasting one day only In 1938 the same fever for 4 days Since the Christmas of 1942 she has had repeated attacks of fever elicited by muscular effort and accompanied by chills as well as pains in the bones of her calves occasionally also in her fore arms and her head (as a cap on her head) When the fever is on the increase she always wants to extend her arms and her legs as if she should want to yawn and she wants to gasp

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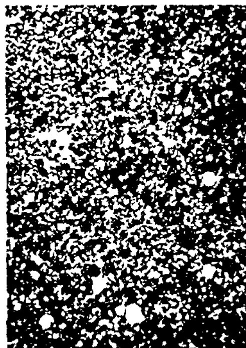


Fig 1 Electron micrograph of a mitochondrial pellet *Most of the mitochondria are of a normal* ultrastructural appearance A few lysosomes and microsomes are seen between the mitochondria

eration the insulin treated patients were given 16 I U of insulin in 1 000 ml of 5 5 % glucose solution by intravenous infusion This was started early in the morning on the day of operation and the solution was infused lowly for about 6 hours Before the operation the patients were given 1 ml of hydromorphine scopolamine and one hour later they were anesthetized with Evipal succinyl choline d tubocurare and nitrous oxide All biopsies were taken as soon as possible after the operation had started

All patients were in a good nutritional condition The diabetics kept to an ordinary Swedish diet but with a restriction of sweet foods.

The muscle specimens were immediately placed in ice-cold 0 25 M sucrose and cut with a pair of scissors The rinsed sample was weighed and homogenized in 5 volumes of the tris-potassium-chloride medium described by Chappell et al 2 The homogenate was diluted with the medium to contain 1 g of muscle per 10 ml and centrifuged for 10 min at 700 × g in a Phywe refrigerated centrifuge The supernatant was then centrifuged for 20 min at 10 000 × g The mitochondrial pellet was washed twice with the tris medium and recentrifuged Finally, the surface of the mitochondrial pellet was washed twice with 0 25 M sucrose and suspended in 0 25 M sucrose All operations were carried out at 0—3° C The mitochondrial suspension was frozen and stored at — 27° C until used for lipid extraction

Electron microscopic examination of different sections of the mitochondrial pellet showed a good purity Fig 1 shows a representative preparation

After lyophilization the mitochondrial suspensions were extracted for 30 min at 50 C in a chloroform — methanol mixture 1 2 (v/v) The lipid extracts were then passed through a glass filter funnel and the residue was washed with the same solvent The solvent was evaporated at reduced pressure in a stream of nitrogen The lipids were redissolved in 20 ml of a chloroform — methanol mixture 2 1 (v/v) Non lipid contaminants were removed by partition against 5 ml of 0 1 % sodium chloride

In order to separate the phospholipids from the neutral lipids the lipid extracts were dissolved in a mixture of 8 % diethyl ether in light petroleum (b p 60—80 C) and pipetted on a silicic acid column (Bio-Rad Silicic acid minus 325 Mesh freshly activated for 12 hours at 110 C) One gram of silicic acid was used for every 20 mg of phospholipids The neutral lipids were eluted with the diethyl ether — light petroleum mixture and the phospholipids with methanol The purity of the two fractions was checked by thin layer chromatography

Methylation of the fatty acids of the neutral lipid and phospholipid fractions was carried out by boiling the lipids under reflux with methanol containing 5 per cent (v v) concentrated H_2SO_4 for 2 hours The mixture was diluted with half the volume of water and the methyl esters were extracted with light petroleum The light petroleum solution was

Table I Cont

Author	Publ	Age	Medication in days	Complications	Fate
Rosenberg	1964 (1)	20*	21?×3	Pulmonary embolism	
	(2)	28¹	21?×3	Pulmonary embolism	
Schatz et al	1964 (1)	39	2 months	Thrombosis left popl art	Amputation
	(3)	25	21 days	Pulmonary embolism + bilateral venous thrombosis (legs)	
	(5)	23	60 days	Bilateral thrombophlebitis of calves	
	(6)	41¹	21 days	Thrombosis left calf	
Sender	1962	24	4×21	Phlebothrombosis right leg	
Staddon	1962	38	5 days	Thrombophlebitis left calf	
Stewart Wallace	1964 (1)	32	22×21	Wallenberg syndrome right side	L
Zilkha	1964 (1)	23	18 days+20	Vascular lesion of brain	L
	(2)	26¹	20 days×5	Hemianopsia (cortical venous thrombosis?)	L

* The coefficient indicates the number of children Figures within brackets indicate miscarriages
L = cerebral lesion

still more cases have been published but to meet the requirements already indicated I had to confine my interest to the 36 instances They have been briefly summarized in table I

As for my unpublished material it consists of 15 cases 11 cases are Swedish whereas the others have occurred in Denmark (1 case) England (2 cases) and Switzerland (1 case) As to the Swedish cases 7 belong to the Uppsala material and 4 have been kindly described to me by colleagues from other hospitals (Professor Tornblom in Umeå Surgeon in chief Linton (of Molndal) Physicians in chief Jonsson (of Sundsvall) and Lublin (of Gavle) The cases from abroad have kindly been reported to me by Professor Senning of Zurich by Head physician Dr Clausen of Odense and by my friend Dr Hagströmer who was in England at the time when the British cases were mentioned in the daily press and who called my attention to these two instances on one of these British cases a brief comment has recently been made in the Lancet (2 548 1965)

The material thus collected will accordingly be represented by 51 instances, 12 of which concerned arterial thrombosis whereas 37 were of venous origin and 3 were characterized as cerebral it being doubtful whether the lesion in question was due to an arterial or to a venous lesion

A brief summary of the various materials is given in table III

It will be seen that out of all 51 cases 12 died Five died from pulmonary embolism one from thrombosis of the superior mesenteric vein (case I of Reed and Conn) and six from occlusion of arteries (in one case internal carotid and its intracranial ramifications in one case the left medial cerebral and the right anterior cerebral arteries in two cases coronary thrombosis — the most instructive Norwegian case described by Hartveit and the case described by Naysmith — in one case the intracranial

TABLE II Survey of material as yet not published where arterial or venous thrombosis occurred during the use of contraceptive preparations. *In all 15 cases are recorded 11 of whom are Swedish* In addition to these 11 cases we have observed several other similar instances since this paper was written

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Case 1 Age 19 Onset 5 days after end of 21 days medication Thrombosis right leg (venography) Mother when 34 thrombosis after cholecystectomy

Case 2 Age 28 Onset after short period of medication Thrombosis left leg + pulmonary embolism Convalescence stormy

Case 3 Age 42? (!) Onset
a) 7 days after start of first 21-day period phlebothrombosis left leg
b) 11 days after onset of next period phlebothrombosis right leg
Her father had a venous thrombosis when 50 his aunt when 30 (died following embolism when pregnant) and the daughter of this aunt at 20

Case 4 Age 44? Started tablets for 21 days Before this period was up thrombosis of the left leg Started next period after 14 days thrombosis of the right hand (arm) Two sisters have had venous thrombosis one sister had thrombosis of her left carotid artery since the age of 43

Case 5 Age 21 Started tablets Aug 10th First symptoms of phlebothrombosis right calf Aug 25th Next cycle of tablets started Sept 4th Symptoms from calf markedly accentuated Sept 20th Venography

Case 6 Age 20 Started tablets Aug 28th last tablet Sept 17th Sept 19th pain and swelling of right calf Started new cycle of tablets Sept 24th Sept 26th pain and swelling of left calf

Case 7 Age 24 Since March 1965 tablets 22 days out of 28 Taken ill Sept 18th Evidence of coronary infarction on admission Sept 21st.

Case 8 (Tornblom) Age 39 Venous thrombosis of right leg 7 days after onset of medication

Case 9 (Linton) Age 29 Medication for less than 2 months Sudden onset of abdominal emergency Gangrene of intestines. Death Arterial thrombosis of coeliac artery

Case 10 (Jonsson) Age 34 Three children aged 16 7 and 3 When pregnant in the last trimester 7 years ago phlebothrombosis left leg Mother phlebothrombosis when pregnant maternal grandmother likewise phlebothrombosis One sister phlebothrombosis without connection with pregnancy or tablets Had been taking contraceptive tablets for 3 months when admitted for pulmonary embolism Eventually she was sent home but returned with a phlebothrombosis of right leg

Case 11 (Lublin) Age 18 Phlebothrombosis of left leg (confirmed by venography) This was June 6th. Had started tablets in March for contraceptive purposes

Case 12 (Senning) Age 35 Two 21-days periods followed by pulmonary embolism 3 weeks later thrombosis Venography confirmed

Case 13 (England) Age 31 Death from pulmonary embolism Used preparation for a few weeks only

Case 14 (England) Age 22 Death from pulmonary embolism Used preparation

Case 15 (Denmark) The Head physician of the Department of Medicine in Odense J Clausen has at present a woman aged 26 under treatment for coronary occlusion She had for some months taken the tablets in question He is going to report the case in detail elsewhere

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part of the right vertebral artery and in one case of the Swedish material thrombosis of the coeliac axis) However, as to venous thromboses pulmonary embolism was noticed in at least 16 more instances and in several of them it was a very close call Nobody knows what handicap may be entailed for these cases in the future in severe instances hypertension of the pulmonary artery may ensue

In arterial occlusion the result was good in one case only (when the left axillary artery was involved), whereas in four other instances the result was amputation (case 1 of Schatz and coworkers (34)) a Wallenberg syndrome (case of Stewart Wallace (38) and two coronary infarctions (the case of Boyce et al (5) and that of our own material case 15 (Clausen)) To these arterial cases may be added my own case 7 Three more cases should be added where a vascular lesion of the brain was recorded although there is some doubt whether it was due to venous thrombosis or to arterial infarction (the case of Lorentz (21) and the two cases described by Zilkha (46))

The duration of the medication with the contraceptive preparation is illustrated in table IV (the same reservation as in table III)

It will be seen that in the majority of venous thromboses and pulmonary embolism the medication had been taken for a brief period only whereas *in 10 of the arterial* incidents medication did not exceed 6 months and was less than that in most instances

Discussion

It should be readily admitted firstly that the present material of in all 51 cases is of limited size secondly, that no controls are available of thrombosis or embolism in women of fertile age not taking oral contraceptives



Fig 1 Venography of case 5 performed in our department of roentgenology (Head Folke Knutsson, M D) The superficial veins are well filled but the main deep trunk is obliterated by a thrombus the tail of which is to be seen at the arrow

TABLE III Survey of the age of the afflicted patients as well as of the localization of the thrombosis/embolism concerned In one case there was both phlebothrombosis of the leg and thrombosis of the axillary artery hence there is one case in common in the venous column and in the arterial column

Age	Venous	Arterial	Cerebral	Total
< 20	2	—	—	2
20–24	14	2	1	17
25–29	8	3	1	11
30–34	3	5	—	8
35–29	4	2	—	6
40–44	6	—	1	7
Total	37	12	3	51
Dead	6	6	—	
Disability	7	5	3	

TABLE IV Correlation between the duration of the medication and the occurrence of thrombosis and the localization of this thrombosis (venous arterial)

Medication in months	Venous	Arterial	Cerebral	Total
<1	17 (1)	2 (1)	1	20
>1<3	12 (2)	2 (1)	1	15
>3<6	6 (3)	6 (3)	1	13
6<12	1	—	—	1
12	1	2 (1)	—	3
Total	37	12	3	51

Fatality within brackets

However, it is in the nature of things that the number of collected cases in any given centre ought to be limited, the largest material hitherto being that of Schatz et al who were able in Detroit to assemble 6 cases within a limited period Two of these cases we have refrained from using, case 2 because the patient had had her leg in a cast for two days whilst the contraceptive treatment was being started, case 4 because of her age Similar consideration made us exclude case 3 of Reed and Conn and cases 2, 3 and 4 of McGowan The otherwise extremely instructive, fatal case I of Leather had to be excluded since the use of the tablets was connected with an (unexpected) pregnancy It is, on the whole, instructive that in so many instances more than one case has been reported by several authors (20, 23, 29 30, 34, 46)

Secondly it is difficult to see how a control material might be collected which was within limits reliable and compatible with that of women subjec

ted to oral contraceptives The only valid comparison would have been between monovular twins and to subject one of two twin sisters to a treatment that was to be withheld from the other one seems beyond human and psychological possibilities One of my own cases, No 3, has been willing to submit herself to a third test with the tablets in question I do not feel such an experiment warranted Objections have been raised, rightly in our opinion, to the comparisons hitherto made at conferences called in to deal with the statistics in this matter

In order to proceed further the following facts should be stressed

1 Arterial thromboses are very rare during the child bearing age, provided that no severe arterial affliction exists such as atheromatosis, arteritis, diabetes or longstanding hypertension No such factors were present in this material as evidenced also by the post mortem of the Swedish case 9 Venous thrombosis is likewise, uncommon if no cardiac disease, no carcinoma (frequently heralded by thrombophlebitis migrans), no pneumonia and no pernicious anaemia is present (1 2 3)

2 In the vast majority of instances the thromboembolic complications are apt to appear early during medication, *although* exceptions occur

3 In my own cases, 3, 4 5 and 6, it was instructive to notice the occurrence on repeated occasions of thrombosis in connection with the medication with contraceptives (for details see p 4) The same may be said about McGowan s extremely interesting case 1 in a woman aged 32, medication was carried out for 8 cycles, starting in January In May she developed a thrombophlebitis and thrombosis in the left popliteal vein Nevertheless, she proceeded during the next 3 cycles to take her tablets but on each occasion there was an exacerbation of pain and swelling of her left leg For the last 2 cycles she had also cramp-like pains in her right calf, very much of the same type as at the onset of the thrombosis in the left one

4 The burden of proof is certainly up to those who maintain that these preparations are harmless, not to those who like us consider that a certain danger cannot be excluded

5 If the tablets are used for contraceptive purposes it should not for one moment be forgotten that we have to do with previously healthy women and that they are entitled to a reasonable margin of safety One may compare the issue to that of vaccination against poliomyelitis It has rightly been maintained that one case of polio in 10 000 inhabitants represents a severe epidemic, one case in 100 000 inhabitants an ordinary epidemic The tragedy entailed by the use of the Cutter vaccine has taught all of us a lesson It is now considered that even a risk of one case of polio from 10^6 vaccinations is not allowed, not even one case out of 10^7 It has been maintained that some 100 000—150,000 women in Sweden have taken the tablets here in question during the last year There are reasons to doubt this figure, evidence being available to the effect that not more than 42,500 women took the tablets for the first six months of 1965 Anyhow, our 11 Swedish cases have been assembled during the time

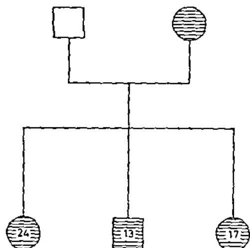


Fig 2 Pedigree of thrombosis in a family described by myself elsewhere (*Bedside medicine* Almqvist & Wiksell Stockholm 1963) The mother had thrombosis in connection with one of her deliveries All the three children had thrombosis in one of the girls in connection with her second delivery at the age of 24 in the son when he at the age of 13 (sic) was operated upon for perforated ulcer and in the youngest daughter when 17 in connection with a miscarriage This family is not described in the present paper but may exemplify the familial appearance of phlebothrombosis

Dec 1964—Sept 1965 It should, moreover be observed that the Swedish material has by no means been systematically assembled 7 cases were recorded here in Uppsala 4 cases were put at our disposal by friendly colleagues who knew of our interest in the matter In view of the evidence already assembled the risk entailed seems to be beyond the margin of safety required

The evidence thus obtained seems suggestive to a point It will moreover serve to stimulate further clinical observations, if possible as suggested by Stewart Wallace on a prospective basis

Perhaps there is another point to be made It has rightly been maintained that women partaking of oral contraceptives may experience symptoms apt to mimic a pregnancy (nausea, tension of the breasts, etc) Whereas opinions have been divergent as to the affections inflicted by these agents on the coagulation of blood (9, 10, 17, 40, 39) recent observations tend to confirm the impression of earlier authors about an increased coagulation ability as in pregnancy Moreover, the blood volume and the dynamics of the venous walls seem to be affected in the same way by contraceptive compounds as by pregnancy It is generally agreed upon that pregnancy entails a certain risk, *although not great, of thrombosis and* embolism One may rightly ask as did Reed and Conn, whether a similarly increased risk is justified where oral contraceptives are concerned

One feels inclined to agree with the two authors just mentioned when they conclude

'If subsequent clinical observations confirm a definite correlation between the use of contraceptive agents taken by mouth and an increased incidence of thromboembolic disease, in our opinion the use of such agents would not be justified in a community in which other methods of contraception are readily available and reasonably effective

If, however, notwithstanding this very fair conclusion the contraceptives nevertheless should be used it seems to be of paramount importance to exclude at least the following women from using them

1 If thrombosis or thrombophlebitis have occurred or are frequent in the family history (2) My own cases 1 3,

and 4, as well as 10 may exemplify this (see also fig 2)

2 If the woman in question herself has had thrombosis or thrombophlebitis on earlier occasions In my own case, 3, such was the case after a miscarriage when 26, although not after normal deliveries when aged 21, 29, and 31

3 Should any disease in itself disposing to thrombosis and embolism be present (p 7) the contraceptives must not be used

4 Earlier liver disorders were noted in the cases described by Reutter et al (hepatitis when aged 12) and by McIntyre et al (jaundice in case 2 when 16)

5 Pronounced obesity should likewise rule out the use of contraceptive compounds

6 Varicosities, present before the administration of the tablets should preclude their use

7 If the patients are in for a tedious sitting, as in travelling by car or by air for say, 8 hours this represents a contraindication This is particularly so if the ladies in question make use of the peculiar contraption called panty girdle, rather a terrific piece of clothing apt to impair the venous drainage from the legs more so in a sitting position

8 The interval between the onset of the medication and the appearance of the thrombo embolic complication makes it at least suggestive that some factor of allergy may be involved This, of course is just a guess but should it turn out to be true allergic patients should be spared these tablets as well

9 Should irregular or undue bleeding warrant the use of these compounds there must be a gynaecological examination before embarking on the tablets lest a severe pelvic disorder should lurk behind the symptoms

10 One has to realize that even with the precautions already mentioned there is apt to remain a risk for thrombosis if this preparation is given to otherwise healthy young women

Summary and conclusion

1 A review is made of 51 cases presenting thrombo embolic complica tions in connection with the administra tion of oral contraceptives 36 cases were derived from the literature and 15 new cases were added 11 of whom were from Sweden

2 There is suggestive evidence about a causal relationship between the com pounds in question and the occurrence of thrombo embolism

3 With Reed and Conn we feel inclined to consider the use of such agents unjustified in a community where other methods of contraception are readily available and reasonably effective At least the conclusion of a coroner in England (Times Sept 7, 1965) regard ing one of the cases here recorded seems warranted It is important that some publicity be given to this not so much as a warning that people should not take contraceptive pills but so they can appreciate that there is a possibility of these unfortunate consequences

4 If for other reasons, the use of these compounds should be allowed there are certain conditions which seem to repre sent contraindications These conditions are briefly enumerated

5 Since this paper was written several more cases have been observed in Sweden collected in part by myself in part by the Royal Medical Board These cases are to be treated in another publication

Addendum

The repeated occurrence of thrombosis in the same patient on different occasions whilst taking the tablets seems to us particularly important as an evidence Another evidence to our mind quite conclusive is the observation recently made in Switzerland by Haefeli and co-workers from the Department of Gynecology in Basel (Gynaecologia *160* 281–292 1965) Ten women who had been using oral contraceptives were operated upon for gynaecological reasons In 5 instances pulmonary embolism occurred in one of them deadly The other five did not obtain any pulmonary embolism, but in two the medication with contraceptives had been stopped 2—5 months before the operation. To have an occurrence of pulmonary embolism in more than 50 % is certainly food for thought. If any female member of my own family applied to me to get oral contraceptives I would most certainly not dare to give it to her

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The Composition of Human Subcutaneous Adipose Tissue in Relation to Its Morphology

By

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The chemical composition of human adipose tissue has previously been studied by a number of investigators The greater part consists of triglycerides and water, while only a few per cent is made up of other lipids or protein (2, 7, 8, 9 10 11 18) Adipose tissue has also been studied with morphological techniques by several authors and cell size and cell number been determined in different conditions (1, 13 20) The relationships between chemical and morphological parameters do not seem to have been studied so far

In the present work subcutaneous adipose tissue from the human has been studied in respect of on the one hand, number of fat cells and on the other hand fat cell size as related to different chemical parameters Such data provide information on the content in adipose tissue of cells other than fat cells This seems difficult to measure directly as will be discussed Such data are also of practical importance If an estimate of the number of fat cells in adipose tissue is needed, the chemical measurements are preferable, because the morphological techniques are considerably more time consuming Furthermore it is not known which cell constituents increase when fat cell size increases

Material

Adipose tissue was taken from the abdominal wall lateral to the umbilicus under local anesthesia or under general anesthesia during operation for gall stones or gastric or duodenal ulcers The body weight index (12) of the patients from whom adipose tissue was taken was between 0 78 and 1 81 None had clinical signs of edema icterus or malignant disease

Chemical methods

Immediately after excision the adipose tissue was placed in Krebs-Ringer bicarbonate buffer with 4 % bovine albumin (Armour, Fraction V) at room temperature dissected into 25—40 mg pieces within 10 minutes after excision blotted on a filter paper and rapidly weighed on a torsion balance It was thereafter extracted with chloro

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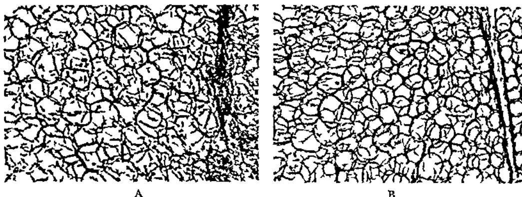


Fig 1 Morphological appearance in 200 μ sections of adipose tissue with mean cell diameters of 125 μ (A) and 80 μ (B)

form methanol, 2 1, (v/v) at least 20 ml extraction fluid per gram adipose tissue by pressing the tissue with a glass rod against the glass wall in the extraction vessel, until the residue had sunk in the fluid The extract was shaken with 1/4 volume of 0 05 M acetic acid The mixture was then allowed to separate into two clear phases In the chloroform phase glyceride glycerol (4) and lipid phosphorus (17) were determined From the glyceride glycerol triglyceride was calculated by assuming a mean molecular weight of the fatty acids of 282 (2) In the tissue residue deoxyribonucleic acid (DNA) was determined according to Webb and Levy (19)

The large amount of triglyceride in the chloroform phase rendered difficult the wet ashing of this extract for lipid phosphorus determination This was therefore facilitated by removing triglycerides chromatographically in the following way The chloroform extract was added to a chromatographic column containing about 1 g of silicic acid (Mallinckrodt activated at 120 C over night) and the neutral lipids were eluted with 2 portions of 5 ml chloroform and then the phospholipids with 2 portions of 5 ml chloroform methanol 1 9 (v/v) Since neither glycerides nor lipid phosphorus were found in the second chloroform eluate this was later excluded in the procedure and since the second 5 ml portion of chlororform methanol contained only an insignificant amount of lipid phosphorus, this was also excluded The chloroform methanol eluted all phospholipids as judged from experiments with repeated chromatographic procedures and essentially total recovery This eluate was evaporated and used for lipid phosphorus determination

Morphological methods

The samples used for morphological studies were immediately chilled to —40° C and then fixed in Zenker formol solution as described by Bjurulf (1) Tissue sections of 200 and 20μ thickness were used the latter stained with hematoxylin eosin

For determination of cell size the technique described by Bjurulf (1) was used with the following minor changes In the 200 μ section the cell diameter was measured in the widest part of the cell in 2 directions perpendicular to each other with the aid of an ocular micrometer The mean of measurements on 50 cells distant from connective tissue bundles and situated consecutively on a straight line in the visual field was used as mean cell diameter

For determination of the number of fat cells per mm³ tissue the 20μ section was used and at least 100 cells counted The number of cells was then obtained by using the formula of Flodérus (6) applied according to Bjurulf (1) except that the height of the smallest cell calotte included in the cell count

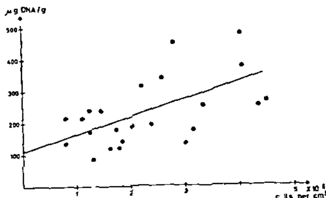


Fig 2 Correlation between adipose tissue content of DNA per g wet weight and number of fat cells per cm³ r 0 54 p < 0 005 y = 0 048 x + 118

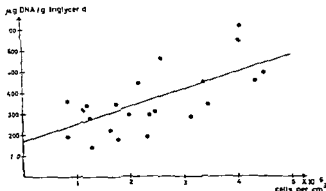


Fig 3 Correlation between adipose tissue content of DNA per g triglyceride and number of fat cells per cm³ r 0 66 p < 0 0005 y = 0 082 x + 170

was taken as one tenth of the mean cell diameter of the tissue in question

The error of the methods in the determination of cell number was determined in 12 samples and found to be about 14 %

Results

Fig 1 shows for 200 µ sections the appearance of two different types of adipose tissue, with large (125 µ) and small (80 µ) mean fat cell diameters

Correlations between number of fat cells per units of adipose tissue volume and different chemical components were investigated Fig 2 shows the relationship between number of fat cells per cm³ and amount of total DNA per units wet weight, thus including DNA from cells other than fat cells A positive correlation was evident ($p < 0.005$) If instead the calculation was performed against amount of DNA per gram triglyceride the regression coefficient and the significance ($p < 0.0005$) were higher as seen in fig 3 Phospholipid phosphorus however, did not correlate significantly with cell number

The contents per cell of different constituents were calculated Here a specific gravity value for adipose tissue of 0 94 was used to convert volume to weight No correlation to cell size was found for

expected Futhermore, the amount of phospholipid was not correlated to cell size either which suggests that membraneous constitutents and cytoplasmic mass (11) do not increase when fat cell size increases The amount of triglyceride, however, increased with cell size

Summary

The amount and size of human subcutaneous adipose fat cells were compared with the contents in adipose tissue of deoxyribonucleic acid, phospholipid phosphorus and triglyceride It was found that the total amount of deoxyribonucleic acid in adipose tissue, including deoxyribonucleic acid from cells other than fat cells, gives a good relative estimate of the actual adipose cell number, while the amount of triglyceride per unit of deoxyribonucleic acid gives a good indication of adipose cell mean size When adipose tissue cells increase in size, the amount of deoxyribonucleic acid seems to be constant as expected The amount of phospholipid, this being an estimate of cytoplasmic mass, is likewise unchanged, while the amount of triglyceride is considerably increased

Acknowledgement

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Peri-collagenous Amyloidosis

A study of 51 cases

By

J Hallén and R Rudin

Many systems have been suggested for the classification of amyloidosis on clinical and patho-anatomical grounds Table I reflects the latest suggestion (14) based on the observation that, when examined in polarised light, amyloid is seen in some cases to be deposited along reticular fibrils, and in other cases along collagenous fibrils (13, 24) During the last 15 years, interest in amyloidosis has shifted to a large degree from the "secondary (peri reticular) to the 'primary (peri collagenous) form Many problems concerning the latter form are still unsolved and further investigations of this rare but serious disease are desirable Below is given an account mainly clinical of 51 cases seen at Malmö General Hospital (M G H)

Material and methods

The material consisted of all cases of peri collagenous amyloidosis demonstrated at necropsy during a 4 year period (1961—64) These cases are divided into 2 groups I senile cardiac amyloidosis — cases with mainly amyloidosis of the heart in subjects above 70 years — and II classical primary amyloidosis and amyloidosis in subjects with myelomatosis Group II included 5 further clinically interesting cases 2 of which were seen before the above mentioned period and 3 subjects from areas not catered for by M G H

In Malmo with a population of about 230 000 inhabitants 60 % of all persons who die are examined post mortem at the Institute of Pathology This is 98 % of all who die in M G H which is the only hospital for physical diseases in the town

The heart lungs liver spleen kidneys and prostate are examined routinely and other organs according to the nature of the case

The stain routinely used is haematoxylin-eosin and if amyloidosis is suspected van Gieson gentian violet MacManus and Congo red Sections stained with Congo red are examined in polarised light

Paper electrophoresis of the serum and urine and immunological classification of M components (30) (pathological proteins (33)) if any were done at the Department of Clinical Chemistry with the methods used by Bachmann and Laurell (3)

Submitted for publication November 22 1965

TABLE I Classification of amyloidosis

Peri reticular		
Secondary	Hereditary	
Chronic infection Osteomyelitis Tuberculosis Bronchiectasis Chronic pyelonephritis Rheumatoid arthritis Malignant neoplasms	Familial Mediterranean fever	Familial urticaria and deafness Muckle & Wells (25)
Most common localization		
Liver Spleen Kidney	Kidney Spleen	Kidney

Case reports

Data given in tables IV and V are usually not repeated in the following reports

Case 1 (G H) Male born in 1895 In 1918 he had been admitted to hospital because of severe joint pain from which he had recovered after 1 month From 1945 on he had had spells of iritis as well as dropsy of the knee joints which were tapped about once a year In 1960 and 1961 he had been admitted to hospital because of severe knee pain and pain in the toes of the left foot attended by local increase in temperature oedema and erythema as well as dropsy of the knee joints R B C 3 5 mill E S R 120 mm/1 hour γ globulin (paper electrophoresis) 3 3 g/100 ml (polyclonal) no L E cells negative Rose Waaler's reaction normal antistreptolysin titre and uric acid in serum 5 mg/100 ml no proteinuria or pyuria and the N P N normal During that period his body weight fell from 73 to 65 kg The symptoms had gradually abated except for hydropsy of the knees

In March 1964 he was admitted to M G H During 6 months he had been troubled by mild symptoms of cystitis thirst, diarrhoea shortness of breath anorexia and fall in body weight to 56 kg He was then cachectic, pale, dyspnoic and with widespread hyperplasia of the lymph nodes of such a degree that malignant lymphogranulomatosis was suspected Apart from exudate in the knee joints he had no joint symptoms In addition to what is given in tables IV and V it might be mentioned that the urinary sediment — examined on three occasions — was normal and the thrombocyte count was 560,000 (1 determination) One week after admission he died in uraemia

Post mortem examination showed besides amyloidosis of the spleen and kidneys deposits of amyloid in the myocardium intestines muscles liver vessels as well as marked plasma cell infiltration of the lymph nodes and pyelonephritis As in the sternal punctate the number of plasma cells in the bone marrow was not increased

Case 2 (E H) Female born in 1903 The patient died at the age of 59 years in 1963 During the 1950s she had occasionally been examined for different trivial conditions In 1951 and 1955 the E S R was 10 mm/1 hour in 1957 it was 20 and 1958 it was 47 mm/1 hour Serum electrophoresis in 1958 showed no abnormalities apart from a

Peri-collagenous				
Myelomatosis	Primary			Hereditary
	Classical primary	Senile cardiac	Local	Rukavina et al. (31) Andrade (1) Frederiksen et al (11)

Most common localization				
Vessels heart respiratory tract tongue intestines skin joints nervous system				

low γ globulin value (0 7 g/100 ml) In 1957 the urine was for the first time found to contain protein The E S R. was unchanged but no proteinuria could be demonstrated after 1960 when Heller's test was replaced by the Albustix method In 1958 she had increasing pain in the hands and fingers especially on exertion and later paresthesia and weakness In 1959 she complained of pain in the chest when walking In May 1962 she was admitted to hospital because of these symptoms and periocular haemorrhages which had in the meantime appeared The tongue was shiny but otherwise physical examination revealed nothing abnormal Paresthesia of the hands could be produced by pressure over the volar carpal ligament. In addition to what is shown in tables IV and V it may be mentioned that the plasma cells in marrow smears were polymorphous and that several of them contained nucleoles. In the autumn of 1962 surgical exploration of the carpal regions revealed large yellow white masses of amyloid which were removed subjective improvement resulted In December 1962 roentgen-examination showed widespread skeletal lesions reminiscent of myeloma. Cytostatic treatment (Melphalan di 2-chloroethyl p-aminophenylalanin) had no demonstrable effect on the clinical picture or the amount of Bence Jones protein in the urine After an attack of symptoms suggesting pulmonary embolism, uraemia supervened During the last week of life she had increasingly severe anginal pain which in the beginning responded favourably to treatment with nitroglycerine but afterwards became resistant and was then attended by an increase of the serum transaminase (G O T 100 U) In January 1963 the E C G which was normal in December 1962 showed episodes of fibrillation, later ventricular extrasystoles and finally a Wilson block The patient died in March 1963

Post mortem examination showed not only extremely widespread amyloidosis but also thrombi in the femoral veins and an old pulmonary infarction The diagnosis of myelomatosis was somewhat doubtful from a patho-anatomical point of view but the clinical picture was clear-cut with plasmocytosis in the bone marrow skeletal lesions and light chain component in the urine

Case 3 (J L) Male born in 1897 Early in 1960 he had had several attacks of cystopyelitis after which he had had constant pyuria In August 1961 the prostate gland

was extirpated because of benign hypertrophy In the Spring of 1961 he had troublesome shortness of breath In August 1961 roentgen examination showed slight enlargement of the heart and the E. C. G showed *low voltage and negative T-waves in leads I* V_5 and V_7 During 1961 he lost 14 kg body weight. In November 1961 he was admitted to M G H. because of a left sided minor stroke Roentgen examination then showed a heart volume of 700 ml/m² and congestion of the pulmonary vessels and fluid in the right pleural cavity He was again admitted in December because of dyspnoea and 3 litres pleural fluid was tapped from the right side At follow up every 1—2 weeks at the out patient department 2—3 litres was drained from the right pleura until he was finally admitted to M G H. in March 1962 because of shortness of breath, weakness, marked loss of body weight, cyanosis, oedema of the legs, tachycardia, blood pressure 95/70 *mm Hg* signs of right-sided pleural effusion and the liver palpable 3 finger breadths below the costal arch. In addition to what is given in tables IV and V it might be mentioned that there were a number of plasmocytic reticulum cells in the bone marrow The patient suddenly died in April 1962

Post mortem examination revealed amyloidosis particularly of the heart pyelonephritic contracted kidneys with papillary necrosis and right-sided encephalomalacia

Case 4 (G L.) fig 9) Female born in *1884* In February 1961 without preceding respiratory tract infection, she became tired and had leg oedema. The output of urine decreased she became subfebrile and for two days *she had slight dull lumbar pain* She was admitted on March 13 with moderate general oedema, *B P 210 110* mm *Hg* and otherwise normal physical condition The E. S R. was 52 mm 1 hour and later rose to 130 mm. The antistreptolysin titre and serum cholesterol were normal She had *oliguria, proteinuria* (about 8 g per day) the first month sometimes slight microscopic haematuria and during the first 2 weeks sometimes a moderate number of hyaline and granular casts. In the course of 5 weeks the N P N fell from about 50 mg/100 ml to a normal level but the oedema increased During percutaneous drainage from May 24 to June 2 the weight curve ceased to rise *From June 4 to June 14 she was given ACTH* (20 I U /day) In the first week of July she began to lose weight progressively Afterwards follow up showed a slow but definite improvement. In 1954 she felt well had no oedema and the blood pressure was 185/100 mm Hg, E. S R. was 43 mm/1 hour the urinary sediment was normal and proteinuria was less marked (0 08 g/100 ml) In 1956 the serum electrophoretic pattern was normal In 1960 oedema of the leg recurred, the blood pressure was 200/100 mm Hg and *the E S R. 52 mm/1 hour In 1961 she died* from cerebral vascular lesion

Post mortem examination which was not so extensive as might be wished showed general arteriosclerosis and a large haemorrhage in the right cerebral hemisphere, as well as an advanced renal amyloidosis of pericollagenous type and deposits in pulmonary vessels too

Case 6 (*T N*) Male born *in 1915 He had* since 1954 been troubled by increasing unsteadiness of gait cramp stiffness and paresthesia of the legs In April 1960 he had shortness of breath and retrosternal pain on exertion and he was admitted to M G H in May 1960 with slight leg oedema but without any other signs of cardiac disease The perception of vibration in the legs and of passive movements of toes was absent while perception of pain, temperature and touch was intact as was postural steadiness The strength of the legs was good but fasciculations were seen in the calf muscles The knee and ankle reflexes were absent, while the tendon reflexes in the arms were normal as was Babinski s sign The cerebrospinal fluid was normal E E G normal. E. C G negative T waves in leads corresponding to the left ventricle Roentgenographs of the heart and lungs revealed nothing remarkable Nitroglycerine had a good effect on the anginous pain the degree of incompensation

TABLE II Cases of peri-collagenous amyloidosis diagnosed during a 4 year period

Year	No of autopsies	Cases of amyloidosis		
		Senile	Primary and myelomatosis	Total
1961	1 220	6	3	9
1962	1 320	5	2	7
1963	1 408	16	2	18
1964	1 464	9	3	12
Total	5 412	36	10	46

varied and the leg symptoms persisted unchanged In July 1961 he was admitted be cause of increased breathlessness His cardiopulmonary condition was as before the liver was palpable three finger breadths below the costal arch and transient bilirubinaemia was noted Roentgen examination general enlargement of the heart (600 ml/m²) and pul monary congestion He left hospital somewhat improved after two weeks treatment with digitalis and was re admitted for the last time on October 1 1961 The unsteadi ness of the legs had increased and they were now tremulous and weak. On September 30 he had got fever (39 C) and severe retrosternal pain He was in pain he had dyspnoea and leg oedema otherwise his condi tion was as before Roentgen examination showed pneumonia like condensation of the right apex and further enlargement of the heart The serum bilirubin was now con tinuously increased (2 4—4 1 mg/100 ml) as was the alkaline phosphatase (13—22 Units Buch & Buch) The G O T was 930 U on the first day after which it began to fall but even after two weeks i was still between 100 and 200 Units The temperature fell, but despite intense dehydration treatment with various thiazide preparations and finally aldosterone inhibitors the patient succumbed to cardiac incompensation

Post mortem examination showed amyloidosis of the heart degeneration of the posterior columns of the spinal cord and subdurally in the thoracic region strange parchment like deposits made up of collagen tissue poor in cells and no amyloid A number of small emboli of obscure origin were found in the peripheral branches of the pulmonary artery The bone marrow was not examined

Case a (A A) Female born in 1918 After bronchitis in 1931 she had asthmatic symptoms periodically and since parturition in 1946 proteinuria of varying degree — most severe at the time of discovery when it was 1 4 g/100 ml. From 1948 onwards she had generalised pruritus Because of this and fatigue loss of body weight and swelling of the legs she was referred to M G H on December 15 1950 The skin was dry and scaly with numerous stearin like papules and patches of dirty brown pigmentation The hair was thin the face and legs oedema tous and the tongue was shiny The thyroid was moderately enlarged and the axillary and inguinal lymph nodes were the size of peas up to hazelnuts firm and not tender to palpation Moderate rhonchi were heard the liver extended 4 finger breadths below the right costal arch and the spleen 4 finger-breadths below the left costal arch In addi tion to the data given in tables IV and V it might be mentioned that W B C was 11—18 000 with up to 7 600 eosinophils thrombocytes 565 000 total serum protein 6 and albumin 2 8 g/100 ml and thymol turbidity formol gel and Takata s reaction were normal Treatment with ACTH had no demonstrable effect on the asthma though the eosinophilia decreased the itching abated considerably and large amounts of urine were

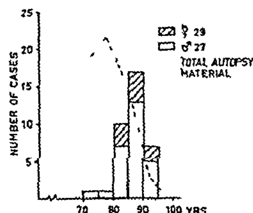


Fig 1 Age distribution of senile amyloidosis and of total autopsy material during a 4 year period

passed In January 1951 the N P N rose and in March the patient died in uraemia

Autopsy showed widespread amyloidosis and bronchopneumonia

Results

Frequency

During the 4 year period in question, 46 of the 5,412 subjects examined post mortem were found to belong to one or the other of the 2 groups mentioned above (table II) The prevalence was 9 cases per 1 000 autopsies, 7 cases per 1,000 of senile cardiac form and 2 per 1,000 of other forms The incidence could be calculated only for "classical" primary amyloidosis and was found to be 1—2 per 230,000 persons per year

Senile cardiac amyloidosis (group I)

The group consisted of 27 men and 9 women The age distribution of these patients showed a shift to the right — higher age classes — compared with that of the entire autopsy series (fig 1) The mean age was 86 years for males as well as for females

In 18 cases the amyloidosis, which was always peri collagenous, had involved only the heart — mostly vessels only — and in 18 also mainly the blood vessels in the lungs, liver, spleen and kidneys *The amyloid deposits* were usually moderate to slight and only in one case did the gross appearance suggest amyloidosis

Coronary sclerosis of varying severity was seen in all of the cases and old or recent infarctions in 8

Fourty six per cent had or had had cancer Of 7 subjects (6 males) with chronic pyelonephritis, two had cancer of the prostate The woman with pyelonephritis had papillary necrosis

None had had rheumatoid arthritis *or chronic infectious diseases apart from* pyelonephritis In many cases (40 %) the clinical picture had been dominated by cardiac incompensation of varying severity *In two cases death had been* caused by acute myocardial infarction Also in the other cases in this group the clinical picture had been dominated by diseases of old age

Twenty one subjects had been examined with E C G As expected from the high frequency of coronary sclerosis, the electrocardiogram had been normal in only two cases

Electrophoresis had been performed during the last 6 months of life in 4 cases and had revealed M components (γ M 1 3 g/100 ml γ G 0 9 g/100 ml) in two The gamma fraction in the others was normal or showed a slight diffuse increase Sera from 5 others had been studied electrophoretically one to three years before death, but no M components had been found Post mortem ex

TABLE III Main sites of amyloid in group II

Case	Heart	Kidney	Liver	Spleen	Lung	Adrenal	Gut	Skin	Fat tissue
1	+	++	V	+++	0		+		
2	+++	+++	V	V	+++	V	+++	+++	+++
3	+++	+++	V	+	+	V		+	+++
4		+++			V				
5	+++	V	V	V	V				
6	++	0	V	V	V	V	V		
7	++	++	V	V	V	V			
8	+	++	0	+	0				
9	V	V	V	V	V			++	
10	V	0	0	0					
a	+	+++	+++	+++	0	+++	++	V	
b	+++	++	V	+++	+++		++	++	++
c	+++	V	V	V	+	V			
d	+++	++	0	+	+	V	0		
e	++	+++	V	+	V	V			

+++ abundant amyloid.
++ amyloid seen in ordinary light microscope
+ amyloid *seen only at examination in polarised light.*
V amyloid seen only at examination in polarised light and only vascular deposits.
0 no amyloid

amination of the bone marrow was suggestive of myelomatosis *in one case, in* which the clinical picture was that of cardiac incompensation presumably the cause of death In that case electrophoresis had not been performed but the E S R and blood values had been normal

Classical primary amyloidosis and amyloidosis in patients with myelomatosis (group II) (All subjects who died between 1961 and 1964 are given numbers the remainder letters In the text and tables the reference numbers and letters of cases in which histories are given are italicized) The amount and the localisation of amyloid deposits are given in table III

Peri collagenous deposits were regular *findings* A more detailed report of the histological findings will be the subject of a future paper by one of us (R R)

The mean age of this group (9 men and 6 women) at death was 62 years and the age at clinical onset in the 11 cases where this could be dated was 49 years

Apart from the three cases with chronic joint symptoms (case *1*) and/or with pyelonephritis (cases *1*, *3*, 9) none of the subjects had had diseases predisposing to secondary amyloidosis

Of unspecific symptoms *loss of weight* had been marked in 10 cases (table IV)

Of the 14 cases of *amyloidosis of the heart* (histological examination had not been performed in case 8) 7 (table IV) had

TABLE IV. Data on cases of peri-collagenous amyloidosis (group II)

Case		Sex	Age at death	Duration of symptoms (yrs)	Weight loss = L oedema = O dyspnoea = D Angina pectoris = A	Blood pressure	Electrocardiogram 2	3	4	5	Heart volume (ml/m² body surface)
1	G H	♀	64	1/4 (?)	L D	115/65	Normal	+			
2	F H	♀	59	6	L O D A	120/80	Fibrillation ES	+	+		>500
3	J L	♀	65	1	L O D	95/70	Fibrillation ES	+	+		>700
4	G L	♀	77	9 1/2	L O	210/110	Normal	+			
5	G M	♀	54	1/4	O D	110/70	Normal	+	+		>500
6	T N	♂	46	7	L O D A	110/80	Normal			+	700
7	S P	♂	48	2	O A[1]	100/80	Normal	+		+	450
8	J P	♂	83	?	L	150/100	Normal		+		
9	K K	♀	79	?		120/60	Normal				
10	H A	♀	93	?		175/90	Normal				
a	A A	♀	32	4	L O	160/90	Normal				Normal
b	B C	♂	52	1	O D	90/65	Normal	+		+	870
c	S E	♀	59	1 1/2	L D	125/80	Fibrillation ES	+			665
d	E H	♀	54	1/2	L A	125/85	ES			+	
e	H H	♂	73	1	L	140/80	Normal			+	625

1 Coronary sclerosis
2 Rhythm — ES ventricular extrasystoles
3 Low voltage
4 Bundle branch block
5 ST-segment depression

had dyspnoea 2 of them (*1* and d), however had had grave anaemia Seven (table IV) had had oedema, severe in case *7* with a nephrotic syndrome but otherwise moderate In one case (case 5) pulsations of the jugular vein had been seen, and 4 (cases *2 3* 5 and *6*) had pleural effusion which in one (case *3*) had repeatedly required thoracocentesis

Three of the subjects (table IV) had had *angina pectoris* in spite of no coronary sclerosis, and responded favourably to nitroglycerine Two had had, shortly before death spells of pain suggestive of myocardial infarction Electrocardiographic examination had revealed no signs of acute infarction, but the glutamine-oxalacetic acid transaminase (G O T) had proved to be increased to 100 Units in case *2* In three patients with extensive amyloid infiltration the G O T level during the quiescent pe

2



3



4



5



6



7



F gs 7

F g Per cul r purp ra n case 2
F g 3 Purpur n a ca f p c lagen s a yl l s s
F g 4 Parcl n l ke subd ral lep s s n ase (
F g M ocar l al a nyl l s s c se (
F g (Four ce ally p l c l a yl l l e r w ll l
F g A p l f on r w p l s cells se

A a M I S and J H ll a l R R l

riod was found to be normal During the later — but not final — stage of the disease, 5 subjects (table IV) had had a systolic blood pressure of at most 110 mm Hg

Electrocardiography (table IV) had re vealed ventricular extra systoles in 4, auricular fibrillation in 3, low voltage in 8, bundle branch block in 4 and ST T depression as in coronary insufficiency in 5 Only in cases 9, 10 and *a* had the E C G been normal

Roentgen examination had shown con siderable enlargement of the heart in 6 of 9 patients with amyloidosis of the heart (table IV) Like cardiac incom- pensation roentgenologically demon strable enlargement of the heart had often developed rapidly, e g in case *6* the volume had increased from 390 to 700 ml in 10 months and in case b from 450 to 870 ml in 6 months

Purpura was noted in cases *2* 9 and c (figs 2 and 3), mainly around the orbits, on the neck on the breasts and the perineum Two (cases *a* and c) had stearin like *papules* (fig 8) and one (case *a*) severe itching and *hyperpigmentation*, which were also seen in cases b and d As judged by the response of the eosino phils to ACTH in case *a* and by the ex cretion of adrenocortical steroids in case b adrenal function had been normal in these two subjects Skin biopsy in case b had shown amyloid as well as an in creased deposition of melanin In case *a* the skin was thick lichenised and dry and hair growth was lacking on the body as well as on the lateral parts of the eye brows

Macroglossia had been noted in only case c while case *2* had a shiny tongue

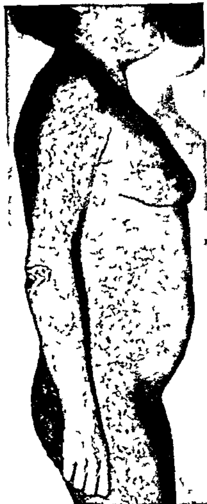


Fig 8 Papules and hyperpigmentation in case *a* with cutaneous amyloidosis

for which the only explanation (hyper normochromic anaemia and B_1 in serum normal) was the massive deposition of amyloid found at post mortem examina tion These two subjects had also had a *carpal tunnel syndrome* In case *2* large polypous masses of yellow peritendinous hyaline tissue had been removed from the carpal region By histological ex amination this structure which had part ly infiltrated the tendons and muscula

TABLE V Data on cases of peri-collagenous amyloidosis (group II)

Case		R B C (mill)	Bone marrow plasma cells (%)	Skeletal X ray osteolytic areas	E.S R. (mm/1h)	Paper electrophoresis serum — g/100 ml		
						Alb[a]	γ[a]	M[b]
1	G H	2.6	1 8		130	2 0	3 5	—
2	E.H	3 1	8 7	+	60	4 5	0 4	—
3	J L.	4 5	5 4	Normal	36	3 3	0 9	1 4 γG
4	G L.	3.2			130	4 2	1 1	—
5	G M	3 9			6			
6	T N	4 5			30	3 4	0 6	—
7	S P	3 9	4 8	Normal	121	1 1	0 4	0 5 γG
8	J P	3 6	2 9	Normal	12	3 2	0 4	0 7 γG
9	K.K.	5 4						
10	H A.	3 7	[c]24 9	Osteoporosis	117	3 3	0 6	2 9 γA
a	A.A.	2 9	0 5		50			
b	B C.	3 9	8 0	Normal	31	3 5	0 7	—
c	S E.	3 3	6 0	Osteoporosis	45			
d	E H	2 8	16 5	+	142	3 6	0 4	9 4 γG
e	H H	3 5	5 2	Normal	55	4 6	0 6	—

[a] Albumin

[b] M-component concentration and immunological type

[c] See fig 6

ure and contained spaces with viscous fluid proved to be amyloid

Myelopathy with signs of lesion of the posterior columns muscular fascicula tion in the calves slight loss of strength and loss of knee and ankle jerks were seen in case *6* The medulla spinalis showed unspecific degeneration partic ularly dorsally Subdurally in the thoracic region there were parchment like deposits (fig 4) made up not of amyloid but of collagen tissue poor in cells

Enlarged lymph nodes were seen in cases *1*, 7 and *a* to such an extent in *1* and 7 that malignant lymphogranulomatosis had been suspected No amyloid deposits were seen microscopically In case *1* there was massive plasma cell infiltra tion of the lymph nodes although the bone marrow was normal, and in the two others there was unspecific hyper-plasia

Anaemia (R B C < 4 mill mm³) usually normochromic was noted in 10 cases table V) The white blood cells were normal in number and distribu-tion except in case *a* with leukocytosis (up to 7 600 eosinophilic leukocytes/mm³) This patient also had pronounced

Urine Protein (g/day)	B J [4]	N P N (mg/100 ml)	Dominating symptoms	Causes of death
2	?	120	Uraemia enlarged lymph nodes	Renal failure
4	+	120	Carpal tunnel syndrome purpura, heart failure angina pectoris uraemia	Heart failure
1	0	47	Heart failure debility	Heart failure
8	?	53	Nephrotic syndrome	Cerebrovasc. lesion
0	0	31	Heart failure	Heart failure
0	?	48	Myelopathy heart failure angina pectoris	Heart failure
10	+	29	Nephrotic syndrome enlarged lymph nodes	Cerebrovasc lesion
3	0	35	Debility	Aortic aneurysm
?	?	87	Uraemia sudden coma	Renal failure
0	0	27	Skeletal pain	Myelomatosis
4	?	65	Itching uraemia enlarged lymph nodes	Renal failure
0	0	42	Purpura carpal tunnel syndrome heart failure	Perforation of the colon
2	?	25	Debility	Cerebrovasc lesion
3	0	41	Skeletal pain angina pectoris	Heart failure
4	+	50	Uraemia debility	Renal failure

[3] Gammaglobulin fraction excluding any M-components

[4] Light chain component.

and constant *thrombocytosis* (> 500 000/mm³) Constantly increased thrombocyte values (> 400 000/mm³) were also noted in case b and in case *1* (one determination), the other 8 cases studied in this respect were normal, except for case 8 in which a count of 330 000/mm³ had been recorded during the last spell in hospital but 390 000—550 000 on 3 occasions one year previously

Bone marrow smears were available from 11 cases In three (table V) the number of plasma cells per 1,000 nucleated cells was less than 3 % and in eight 5—17 % Pronounced polymorphism and atypia had been found in two cases (10 and c) and moderate in three (*2*, *3* and d) In case 10 (fig 6) 25 % of the bone marrow cells had one or two round or oval nuclei with a coarse chromatin The nuclei, which were seldom eccentrically displaced were surrounded by pale cytoplasm usually sparse Most of the very large plasma cells in case c had nucleoles (fig 7) In none of the cases were plasma cells seen with flaming, intranuclear inclusions or compartment formation (29) No amyloid had been found in the bone marrow

Roentgen examination of the skeleton

(table V) showed osteolytic lesions in two cases and osteoporosis in two

The E S R (table V) was normal in only one case *Paper electrophoresis* of the serum had been done in 11 cases (table V) In 8 cases the albumin concentration had been reduced No extra components had been seen in the α_2—β_1-region (31) Eight subjects had had hypogammaglobulinaemia and one polyclonal hypergammaglobulinaemia (3 5 g/100 ml) Five patients had an M component (four γ G one γ A) in the serum, and in two of them the concentration was high (2 9 and 9 4 g/100 ml, respectively) One of these as well as two others had had a light chain component in the urine (table V) The occurrence of such a component in the urine may be regarded as excluded in 4 cases in two (5 and 6) where no protein could be demonstrated by Heller s test and in two (3 and d) in which this test had been positive but electrophoresis of the urine had shown no M component In the remaining 4 cases urine electrophoresis had not been done or the urine had been examined only with Albustix which does not exclude the possibility of an M component (15)

In 10 cases the N P N had been more or less increased (table V) Albuminuria had been demonstrated in 10 cases Despite renal amyloidosis proteinuria had not been demonstrable in case b

The *diagnosis* had been established ante mortem in 4 cases and suspected in one (case 7) In case *a* in which amyloidosis had not been suspected the disease had been discovered at examination of a liver biopsy specimen in case b the condition had been suspected and had been confirmed by later gingival biopsy, and in cases *2* and c the diagnosis had been based on the clinical picture

In addition to the cases described here we have had the opportunity of diagnosing amyloidosis before death in two patients referred to us from other hospitals One was a woman with typical cutaneous bleedings (fig 3) M-component in the serum and skeletal lesions The other was a man with obscure rectal haemorrhages and a serum M-component Deficiency of coagulation factor X confirmed the suspicion as did later post mortem examination This case will be described separately in a future paper (27)

The interval between the onset of symptoms and death was three months to 9 1/2 years (average two years and 10 months) (table IV) Amyloidosis of the heart and/or kidneys had been the main clinical cause of death in 9 cases One of the patients had died from perforation of the amyloid infiltrated colon In the other cases the role played by the amyloidosis in the causation of death is obscure or irrelevant

In 1965 two cases with M-components have so far been found to have cardiac amyloidosis at autopsy The 80-year-old man had had a γ A-component (0 8 g/100 ml) since 1959 when he had been admitted for precardial pain Neither bone marrow smears and skeletal X rays nor autopsy gave evidence of myelomatosis The cause of death was cerebral haemorrhage The 84 year-old woman was admitted for heart failure Achalasia had been known for 10 years For a decade she had had a Raynaud syndrome and was now found to have a cold agglutinin (1 16 400 and an M-component (γ M 0 8 g/100 ml She deteriorated rapidly and died one month after admission Both cases fit into the group senile cardiac amyloidosis.

Discussion

The *classification* suggested by Heller et al (14) was slightly modified only to suit the present material, which presumably included no instances of hereditary amyloidosis The name senile cardiac amyloidosis was used to designate cases of a type described previously (7, 10 16 17, 20 26), and the epithet "cardiac was used because the heart was regularly examined but many other organs, e g the seminal vesicles or ductus deferens (12) were not

It was sometimes difficult to classify cases only according to the clinical findings and to the organs affected In some cases it was difficult to distinguish primary from secondary amyloidosis and in others to decide where to draw the line of distinction between the two groups described

One man (case *1*) had, for example a long history of joint symptoms These symptoms were not quite typical of rheumatoid arthritis and Rose Waaler s reaction was negative, but recurrent iritis and polyclonal hypergammaglobulinaemia suggested collagenosis The long duration of the symptoms argued against joint amyloidosis, at least as a cause of the symptoms at onset The final picture with hyperplastic lymph nodes infiltrated with plasma cells indicated that it was some condition other than rheumatoid arthritis with complicating amyloidosis, and post mortem also showed widespread amyloidosis of pericollagenous type

As to a 79 year-old woman (case 9) with uraemia we only know that she is said to have felt well until she was admitted in a state of coma and soon died The chronic pyelonephritis should warrant assignment to secondary amyloidosis', but the amyloid, which was confined mainly to the blood vessels, was of peri collagenous type and chronic urinary tract infection was seen frequently in a recently published series of primary amyloidosis (28)

The case 8, with only isolated renal amyloidosis and M component in the serum was also difficult to classify, but no precipitating diseases were known and the deposits were peri collagenous

In view of the patients ages (83 and 79 years) cases 8 and 9 ought to be assigned to the group senile amyloidosis Since they had no cardiac amyloidosis they were however assigned to the group "classical primary amyloidosis A 93 year old woman (*case 10*) had myelomatosis, but the distribution of amyloid was that of senile cardiac amyloidosis These cases show that also the distinction between the two groups here discussed can be difficult to make

Co existent diseases The frequency of cancer in our first group may seem high It did not differ substantially from that in corresponding age classes in the entire autopsy material of the Institute of Pathology

The significance of pyelonephritis (7 cases in group I and cases *1 3* and 9 in group II — three with papillary necrosis) is difficult to evaluate It has been reported in high frequency in primary amyloidosis and is thought to be causal (28) Pyelonephritis like pictures and papillary necrosis have been observed in renal amyloidosis in certain inbred strains of mice and interpreted as sec

ondary to deposition of amyloid (9) Such a connection might be assumed in case *1*, for example, while in other cases the amyloid deposits were too small (only observable in polarised light) to be the cause of the pyelonephritis

Disturbed plasma-cell activity The connection between myelomatosis — especially with associated Bence Jones protein — and amyloidosis was pointed out by Magnus Levy (23) Kyle and Bayrd (19) often found signs of disturbed plasma cell activity (M-components in serum and/or urine, bone marrow plasmocytosis) in a material of patho anatomically verified primary amyloidosis Ossermann (28) found M components to be even more common in the serum and/or urine among 27 patients corresponding to our group II In our group II similar findings were made in 7 cases while the occurrence of M component in the serum or the urine could be excluded with certainty in only one (*b*)

Understandably only a few of the subjects in group I had been examined with serum-electrophoresis The frequency of M-components increases with age (2), hence the finding of such a component in this old age group is not surprising The finding of such pathologic electrophoretic components in two of 4 patients examined not too long before death and in one with bone marrow plasmocytosis however, suggests a connection also between senile cardiac amyloidosis and disturbed plasma-cell activity Retrospective examination in polarised light of a large series of cases with M-components without myeloma might help to check this possibility

The diagnosis myelomatosis was firm in 3 cases (*2*, *10* and *d*), there being M components, increase in the number of plasma cells in the bone marrow and skeletal lesions, and was probable in case *c*, in which the electrophoretic pattern (1951) could not be judged with certainty, but in which osteoporosis and profound changes in the bone marrow had been seen

In contrast to what Zlotnik and Tal (34) found in experimental amyloidosis, in 11 of the present cases no flaming plasma cells were seen in the bone marrow

The clinical findings in primary amyloidosis have been described in detail by several authors (5, 6, 18, 19, 31) and therefore only a few of the findings will be commented upon here

Amyloidosis can cause angina pectoris (21) What precipitated the serious final attacks of pain in some of our cases is unknown Neither coronary occlusion nor infarction was observed at necropsy

Despite the histological picture of destruction of muscle cells deposition of amyloid in the myocardium did not usually appear to cause any leakage of transaminase Such leakage was demonstrated in only one case after final attacks of pain

Apart from the muscular fasciculation case *6* showed only signs of lesion of the posterior columns in good agreement with the patho-anatomical picture The nature of the parchment like subdural deposits (fig 4) is obscure They did not correspond to the perispinal amyloid deposits seen in Portuguese amyloidosis (8) a hereditary form of amyloidosis (1) with a malignant course showing severe

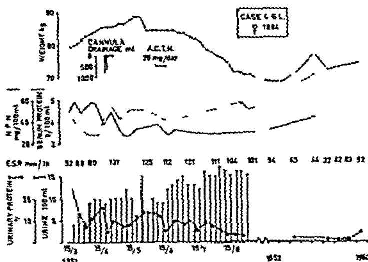


Fig 9 Remission of nephrotic syndrome in case 4

intestinal symptoms mainly constipation and diarrhoea and neuropathy usually starting in the legs

The significance of thrombocytosis in cases *1*, *8* *a* and b is difficult to judge In surveys or reports of primary amyloidosis (5 6 18 19 31) nothing is mentioned about increase in the number of thrombocytes In case *a* the combination asthma eosinophilia and thrombocytosis strongly suggested polyangitis nodosa but subsequent autopsy had revealed no typical changes of this disease or any signs of fibrous healing as sometimes seen in this condition after corticosteroid therapy (4)

The material showed that primary amyloidosis of the kidneys can cause varying clinical pictures as shown previously (22 Uraemia may occur in the absence of a nephrotic syndrome which can persist for several years without supervening uraemia Proteinuria need not occur despite involvement of the kidneys as has been observed earlier by secondary amyloidosis by H Waldenström (32)

Remission of nephrotic syndrome probably due to amyloidosis was noted in case *4* and has been described previously (22)

It is debatable whether the attack of nephrosis in case *4* had been due to amyloid. Post mortem examination 10 years later had revealed amyloid in glomeruli and vessels and nephrosclerosis The patient s age absence of infection in the history normal anti streptolysin titre and the early well developed nephrotic syndrome argue against the possibility of nephritis Sporadic microscopical haematuria, granular casts increased N P N increased blood pressure the rapid development at onset and the slow but complete recovery are all compatible with a diagnosis of amyloidosis The ACTH therapy cannot be regarded as having had any influence on the course of the disease The decreasing N P N during the first 6 weeks with somewhat increasing output of urine speak for some degree of spontaneous remission, and the diuresis and loss of body weight was negligible until about one month after the beginning of ACTH treatment fig 9

The prognosis is otherwise gloomy. Especially when symptoms of cardiac incompensation intervene, the prospects of survival for one year are small. One patient (2) had a carpal tunnel syndrome and angina pectoris for 6 and 4 years respectively before death. When the symptoms from the hands started, hypogammaglobulinaemia was diagnosed, and about one year before proteinuria of a type (negative result with Albustix test) which makes it probable that a light chain component existed already at that time. The final stage was ushered in by symptoms of incompensation and uraemia.

The diagnosis is rarely made ante mortem (31). The possibility of establishment of the diagnosis by the clinicians surely varies considerably with the composition of the material. Eliot et al. (10) reported 20 cases of amyloidosis of the heart in which none was diagnosed ante mortem while Kyle and Bayrd (19) reported a clinical diagnosis in 77 % of 81 cases in which several of the patients had myelomatosis and many had characteristic symptoms.

Summary

A series consisting of 51 cases of pericollagenous amyloidosis is described. Thirty six may be regarded as cases of senile cardiac amyloidosis, 11 of "classical primary amyloidosis and 4 of amyloidosis in myelomatosis.

The prevalence was 9 cases per 1 000 autopsies in a town with an autopsy frequency of 60 %, i.e. 98 % of all patients dying in hospital. The incidence of classical primary amyloidosis was 1—2 per 230,000 persons per year.

The difficulty in classifying certain cases simply according to the clinical picture and which organs were affected is stressed.

As in other series, a correlation was found between disturbed immunoglobulin synthesis (M components in serum and/or urine, bone marrow plasmocytosis) and classical primary amyloidosis. There was evidence to suggest such a correlation in the group senile cardiac amyloidosis too. The appearance of the plasma cells varied considerably from case to case. No flaming plasma cells were seen in the bone marrow smears from 11 cases.

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The Effect of Some Ovulation Inhibitors on the Lipid Metabolism

Preliminary Report

By

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The contraceptive effectiveness of the synthetic steroidal ovulation inhibitors is well documented (2) Their mechanism of action probably involves a multiple target effect The preparations currently in use consist of two components, a gestagen combined with an oestrogen The oestrogen is either 17α ethinyl oestradiol 17β or its 3 methyl ether, whereas the gestagen may vary considerably in chemical structure In principle, most of them are Δ^4 estrenolones (norethisterone, norethisterone acetate, ethynodiol diacetate) $\Delta^{5(10)}$ estrenolone (norethynodrel) or 17α hydroxyprogesterone derivatives

These highly active agents although almost equivalent as regards contraceptive effect might exhibit variation with respect to other biological properties Some of the gestagens used e g, norethynodrel and norethindrone, are metabolized into oestrogenically active compounds (4) This circumstance might indicate a differentiated action on a number of homeostatic mechanisms as compared with the effects of for instance, hydroxyprogesterone derivatives In the search for compounds most suitable for long term use it thus appears important not to regard the contraceptive preparations as a homogenous group of substances

In an attempt to approach the problem from this angle two preparations, norethisterone acetate with ethinyloestradiol (Anovlar Schering) and megestrol acetate with ethinyloestradiol (Volidan BDH), have been compared with respect to the effect on e g liver function glucose tolerance thyroid function, and lipid metabolism With particular respect to the effects on individual plasma phospholipids some findings have been made, which form the basis for this preliminary communication

Submitted for publication December 9 1965

TABLE I Summary of results

	Volidan				
	Before treatment		During treatment		
	1 n = 5	2 n = 5	1 month n = 5	3 months n = 4	6 months n = 3
Free fatty acids (mM)	0 41 ±0 055	0 48 ±0 078	0 47 ±0 065	0 48 ±0 031	0 36 ±0 032
Triglycerides (mg/100 ml)	98 ±19 5	83 ±9 3	111 ±12 9	116 ±19 1	126 ±23 9
Cholesterol (mg/100 ml)	226 ±10 7	218 ±24 4	215 ±19 7	239 ±31 2	212 ±20 5
Phospholipids (mg/100 ml)	255 ±8 9	242 ±17 5	280 ±19 6	306 ±32 3	283 ±19 4
Lysolecithin (% of P lipids)	6 9 ±0 51	6 7 ±0 44	4 1 ±0 42	4 3 ±0 59	4 2 ±0 56
Sphingomyelin (% of P lipids)	22 1 ±1 56	22 2 ±0 67	21 4 ±1 01	20 9 ±0 82	20 1 ±0 60
Lecithin (% of P lipids)	68 1 ±1 12	68 0 ±0 71	70 4 ±1 08	71 0 ±0 98	71 6 ±0 13
Phosphatidylethanolamine (% of P lipids)	3 0 ±0 13	3 1 ±0 27	4 0 ±0 13	4 0 ±0 37	4 2 ±0 12

Mean plasma lipid concentration ± SE before and during treatment with Volidan or Anovlar

Materials and methods

The material comprises ten healthy women, aged 45 to 25 years They all received the ovulation inhibitors for contraceptive purposes

Fasting blood samples were obtained on the 8th and 23rd day of the menstrual cycle before treatment was started The patients then received either Anovlar or Volidan for 21-day periods Fasting blood samples were taken after one three and six months of treatment The plasma levels of free fatty acids (FFA) triglycerides cholesterol and total and individual phospholipids were determined as described previously (5 8)

Results

The results up to date are summarized in table I Neither Volidan nor Anovlar provoked any significant changes in the plasma levels of FFA triglycerides cholesterol, or total phospholipids Volidan induced a pronounced and statistically significant decrease of the percentage of lysolecithin already after one month s treatment ($0 001 < P < 0 01$) This reduction was persistent even after 3 and 6 months A statistically significant increase in the percentage of phosphatidyl ethanolamine was also noticeable after one month on Volidan ($0 01 < P < 0 05$) and was still recognized after 6 months of treatment No significant change was seen in the other phospholipid fractions The patients treated with Anovlar showed no statistically significant change in any of the phospholipid fractions

Anovlar				
Before treatment		During treatment		
1 n=5	2 n=5	1 month n=5	3 months n=5	6 months n=3
0 41	0.52	0 43	0 39	0 42
±0 069	±0 095	±0 038	±0 040	±0 062
73	61	60	70	88
±12 9	±6 9	±4 7	±11 1	±22 0
228	237	192	189	203
±27 7	±31 6	±19 7	±17 9	±25 9
258	258	222	223	245
±21 1	±21 5	±10 9	±15 6	±29 8
7 6	7 1	6 4	6 2	7 6
±0 50	±0 36	±0 44	±0 31	±0 69
20 6	21 6	21 7	21 3	19 4
±0 44	±0 57	±0 63	±0 53	±1 68
69 0	68 5	68 6	69 5	70 0
±0 37	±0 41	±0 66	±0 58	±1 28
2 9	2 9	3 3	2 9	3 0
±0 21	±0 18	±0 28	±0 31	±0 38

Discussion

The present investigation indicates that Volidan causes a strong and statistically significant reduction of the lysolecithin concentration in plasma of a magnitude similar to that observed during pregnancy (6 8) and after the administration of oestradiol 17 to ovariectomized women (7) Other changes in the plasma lipids observed during pregnancy or after treatment with oestradiol were not found to be induced by Volidan under the present experimental conditions A decrease of the plasma lysolecithin concentration has also been demonstrated in patients with liver disease (1 3) The present patients showed no signs of altered liver function as far as can be judged from analyses of bilirubin concentration the thymol turbidity test, and alkaline phosphatase GOT and GPT activity In contrast the other preparation investigated Anovlar had no statistically significant effect on the plasma lysolecithin concentration or on any other parameter studied

The two preparations are identical with respect to the oestrogenic component both as regards quality and quantity but differ as regards the gestagenic factor Anovlar which has so far had no effect on the plasma lipids contains a 19 nortestosterone derivative whereas the active progestin constituent of Volidan is derived from 17α hydroxyprogesterone It would thus

seem probable that the difference in metabolic effect is related to the gestagenic component

It is premature to draw any conclusions from the results presented here as to the clinical suitability of any of these preparations It is impossible to tell whether a lack of response of the plasma lysolecithin concentration indicates a suitable property or whether an effect in this respect signifies the activity of a desirable compensatory mechanism Further work will be carried out along these lines

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Natriuresis and Kaliuresis Following Sodium Chloride Load in Aortic Coarctation

By

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Exaggerated natriuresis is known to occur in subjects with essential hypertension after infusion of hypertonic sodium chloride (1 3) On the other hand normal or diminished natriuresis following sodium chloride infusion has been observed in subjects with renal hypertension (8) Hypertension in aortic coarctation differs in several respects from hypertension due to other causes The mechanism of high arterial pressure in coarctation is still a matter of dispute and evidence favoring both a mechanical and a renal origin of coarctation hypertension has been presented (9) It seemed useful to study the pattern of sodium potassium and total solute excretion after infusion of hypertonic sodium chloride in subjects with aortic coarctation

Methods

Eight adult patients with aortic coarctation were investigated In all cases coarctation was of the usual post subclavian type with only trivial constriction of the left subclavian artery Diagnosis was based upon the typical clinical picture electrocardiogram chest X rays and except for one patient (P A), findings at the operation No symptoms or signs of heart failure were noted in any patient Each subject s age sex weight arterial pressures (by sphygmomanometer) and a brief note on any complicating diseases are given in table I In one subject (H S) the investigation was repeated after surgical correction of the coarctation

The control group consisted of six subjects Four of them had a congenital heart disease with no or only minimal hemodynamic alteration and two of them (S K S L) a significant atrial left to-right shunt Diagnosis was based upon routine examinations and right heart catheterization There were no cases of ostium stenosis and no symptoms or signs of heart failure were noticed The chief features of the control group are given in table II

In neither group was significant renal disease observed so far as could be judged from the clinical symptoms and signs the urine specific gravity protein content and sediment and the serum creatinine level

All infusion tests were made in the fore noon Prior to the day of infusion the subjects were allowed to move freely in the ward and to eat the usual hospital diet Between 7 and 8 a m on the day of infusion each subject drank 10 ml of water per kilogram

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TABLE I Clinical findings of the coarctation group

Case	Sex	Age (yrs)	Weight (kg)	Arterial pressure (mm Hg) Right upper extremity	Left upper extremity	Lower extremity	Complicating diseases
P.A.	♀	35	62.5	190/120	190/120	0/0	Turner's syndrome
S.A.	♀	25	54.5	172/80	170/80	120/100	Aortic hypoplasia
I.P.	♀	33	53.0	215/105	215/105	115/100	—
S.P.	♀	29	79.0	210/105	195/110	150/140	Subacute pyelonephritis
A.F.	♂	41	68.5	200/100	170/90	130/100	—
E.L.	♂	28	73.0	1[illegible]0/80	150/80	130/80	Mitral aortic insufficiency
Fr.I.	♂	31	75.5	180/105	180/105	0/0	Seropositive syphilis
H.S.	♂	34	74.5	220/100	210/100	150/100	—
(H.S. post operative)	♂)	35	81.5	(180/90)	(195/90)	(180/100)	(—)

TABLE II Clinical findings of the control group

Case	Sex	Age yrs	Weight kg	Arterial pressure (mm Hg)	Diagnosis
M.L.J.		28	54.4	125/70	Patent ductus arteriosus
S.K.		28	64.2	125/85	Atrial septal defect (moderate)
S.L.		31	56.5	120/80	Atrial septal defect (moderate)
R.S.		18	61.5	130/80	Wolff-Parkinson-White syndrome
M.S.	♂	19	64.0	130/80	Morbus Roger
E.S.	♂	33	83.0	120/80	Morbus Roger

of body weight at an even rate to ensure adequate water diuresis. The infusion consisted of 15 ml of 2.5 per cent sodium chloride solution per kilogram of body weight which was given intravenously at an even rate between 9 and 10 a.m. Thus each subject received 6.4 mEq of sodium chloride per kilogram of body weight.

Venous blood samples for the serum sodium and potassium determinations were taken with minimal compression at the beginning (A) and at the end (B) of infusion. The voided or, when necessary, the catheterized specimens of urine for sodium, potassium and osmolality determinations were collected over a period of 30 hours: the first (1) from 6 p.m. to 6 a.m. before the infusion morning, the second (2) from 6 a.m. to 9 a.m. in the morning of infusion, the third (3) from 9 a.m. to 10.30 a.m., the fourth (4) from 10.30 a.m. to 12 a.m. after the infusion, the fifth (5) from 12 a.m. to 6 p.m. and the

sixth (6) from 6 p m to 6 a m The sodium and potassium concentrations were determined with a flame photometer (Beckman No 4100) and the urinary osmolality was measured with an osmometer (Advanced Instruments Inc model 63 31)

The t test was used in the statistical analysis

Results

The urine specimen 1 was considered to represent the basal situation The means of the basal excretion rates of the urinary osmols, sodium and potassium were not significantly different between the coarctation and control groups ($P > 0\ 05$)

The means of the excretion rates of the urinary osmols, sodium and potassium of the coarctation and the control groups during the observation period are shown in fig 1 The mean excretion rates of all three parameters were maximal during the first three hours after the infusion The increased excretion of osmols and sodium was still going on at the end of the observation period, while the excretion rate of potassium had already reverted to basal values The increment of potassium excretion occurred when the excretion rate of sodium was maximal

Excretions of urinary sodium and potassium during the first one and a half and three hours after the beginning of the infusion are shown in table III The mean sodium excretion in three hours was significantly less in the coarctation than in the control group ($P < 0\ 05$) No significant difference in the means of the coarctation and the control groups was observed in the one and a half hour excretion of sodium or in the potassium excretion ($P > 0\ 05$)

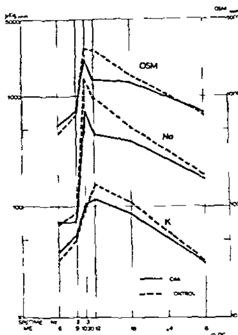


Fig 1 The mean rates of urinary osmol sodium and potassium excretions in the coarctation and control groups

The excretions of sodium expressed as a percentage of the sodium chloride load during 1 5 3, 9 and 21 hours after the beginning of the infusion are shown in table IV The excretion percentage at one and a half hours was not significantly different between the coarctation and control groups The excretion percentage at three hours was significantly lower in the coarctation group ($P < 0\ 05$) The significance of this difference was increased at nine hours ($P < 0\ 02$) and still further at 21 hours ($P < 0\ 01$)

The increase of the serum sodium concentration during the infusion was (mean) 7 4 mEq/l in the coarctation

TABLE III Excretions of sodium and potassium in the urine during the first 1.5 and 3 hours after starting sodium chloride infusion in the coarctation and control groups

Case	U_{Na} (mEq/1.5 h)	U_{Na} (mEq/3 h)	U_K (mEq/1.5 h)	U_K (mEq/3 h)
P.A	42.6	67.8	6.6	18.0
S.A	44.3	73.5	13.5	21.4
I.P	106.5	124.0	10.6	18.4
S.P	73.8	102.6	10.8	16.2
A.E.	31.7	68.5	9.5	21.2
Ee.L.	70.5	121.0	10.0	22.2
Er.L.	100.0	190.0	11.5	29.5
H.S	48.9	88.1	5.5	15.6
	Mean 64.8	Mean 104.4	Mean 9.7	Mean 20.3
	SD 27.65	SD 41.12	SD 2.59	SD 4.44
M.-L.J	48.8	98.0	6.0	14.6
S.K	40.7	83.9	4.9	11.6
S.L.	70.0	135.5	8.2	17.7
R.S	165.0	303.0	14.4	32.4
M.S	122.0	251.0	10.6	33.7
F.S	144.5	251.5	6.3	33.4
	Mean 98.5	Mean 187.1	Mean 8.4	Mean 23.9
	SD 52.54	SD 92.64	SD 3.56	SD 10.34
P for the difference between the means	*0.01*	*0.02*	*>0.05*	*>0.05*

group and (mean) 7.5 mEq/l in the control group. The difference of these means was not significant. The serum potassium concentration increased in both groups by (mean) 0.35 mEq/l.

Discussion

The present study reveals in the coarctation group as compared with the control group a significantly smaller renal sodium excretion – expressed in milliequivalents and as a percentage of the infused sodium chloride load – after three hours following an intravenous infusion of sodium chloride. The excretion of sodium in the early phase just after infusion was not significantly different between the two groups. No significant difference in the renal potassium or total osmol excretion between the coarctation and control groups was observed. The changes of the serum sodium and potassium concentrations following sodium chloride infusion were not significantly different between the two groups.

The exaggerated natriuresis in essential hypertension after infusion of hypertonic sodium chloride seems to be a fairly regular phenomenon that occurs during the first three or four hours after infusion, provided there is no significant renal damage (1, 3). Even non-specific

TABLE IV Excretions of urinary sodium as a percentage of the sodium chloride load during 1.5, 3, 9 and 21 hours after starting infusion in the coarctation and control groups

Case	$\frac{U_{Na}\ 1.5\ h}{Na_{infused}} \times 100$	$\frac{U_{Na}\ 3\ h}{Na_{infused}} \times 100$	$\frac{U_{Na}\ 9\ h}{Na_{infused}} \times 100$	$\frac{U_{Na}\ 21\ h}{Na_{infused}} \times 100$
P A	10.9	17.4	43.3	64.8
S A	13.0	21.6	62.7	106.0
I P	32.1	37.4	73.0	100.0
S P	15.0	20.8	64.6	89.0
A E	7.4	16.1	51.4	86.0
Ec L	15.5	26.6	69.2	95.5
Er L	21.3	40.5	89.5	110.0
H S	10.5	18.9	57.0	73.6
	Mean 15.7	Mean 24.9	Mean 63.8	Mean 90.6
	SD 7.81	SD 9.25	SD 14.12	SD 15.60
M L J	14.3	28.8	80.5	130.0
S K	10.2	21.0	59.0	92.0
S L	19.9	38.5	79.5	122.0
R S	43.0	79.0	126.0	158.0
M S	30.5	62.7	126.0	157.0
E S	28.2	48.6	94.5	128.0
	Mean 24.4	Mean 46.4	Mean 94.3	Mean 131.2
	SD 12.01	SD 21.68	SD 27.07	SD 24.56
P for the difference between the means	>0.05	<0.05	<0.02	<0.01

stimuli such as mannitol and glucose infusions, vasopressin administration (2) iso oncotic fluid loading (13) and change from erect to recumbent posture (7) cause a natriuresis in essential hypertension that exceeds that seen in normal subjects The mechanism of abnormal sodium excretion in essential hypertension has recently been investigated by Hanenson et al (5) From their results of renal function studies it seems probable that hypertension per se is responsible for the exaggerated natriuresis and that there need not be a change in the renal tubular cell metabolism The suggested explanation for the natriuresis is an increase in blood flow through the renal vasa recta secondary to the renal arterial hypertension which could cause an exaggerated natriuresis by removing solute from the extracellular fluid of the renal medulla, thus impairing the efficiency of the countercurrent mechanism In agreement with this hypothesis, normal or subnormal natriuresis has been observed in subjects with hypertension due to renal artery stenosis (6, 8)

Somewhat puzzling is the occurrence of exaggerated natriuresis in some forms of heart disease

Toor et al (12) found the percentage of sodium excretion after sodium load to be higher in pulmonary hypertension and intraventricular hypertension, whether right or left even without any signs of systemic hypertension They think that changes in urinary sodium excretion of these patients may be triggered via receptors in the ventricles

The hypertension in aortic coarctation differs by its distribution and benign nature from hypertension due to other causes In arteries below the coarctation the systolic pressure is similar to or slightly below the range seen in normal subjects in the same arteries while the diastolic pressure is usually above the normal range The upstroke time of the arterial pulse wave below the coarctation is prolonged (9) In these respects aortic coarctation could behave like a renal artery stenosis by diminishing the renal arterial and possibly arteriolar pressure and pulsatile flow and, if the above hypothesis is followed a normal or subnormal natriuretic response to the sodium chloride load could be anticipated

In typical cases of aortic coarctation however the changes in renal hemodynamics should be fairly small A slight decrease of the renal blood flow has been demonstrated in aortic coarctation the flow reverting to the normal range after surgical correction (3) Our results show no significant difference between the coarctation and control groups in the sodium excretion during the immediate post infusion period although the values in the coarctation group have some tendency to be lower Later the sodium excretion becomes progressively and significantly smaller in the coarctation group Thus the pattern of sodium excretion in aortic coarctation differs strongly from the pattern seen in essential hypertension, where the maximal change in sodium excretion from normal values occurs soon after infusion and in the opposite direction Although the basal excretions of sodium in the coarctation and control groups were not significantly different, perhaps due to the small size of our sample, the clear increase of the post operative natriuresis in one of the subjects (H S) suggests the possibility of a pre-operative dampening of the sodium excretion This is in agreement with the findings of Timmis and Gordon who demonstrated a significant post-operative increase of the urinary sodium clearance in seven children with aortic coarctation, which was attributed to the normalized renal pulse wave and pulse pressure pattern (11)

From the studies of Hanenson et al (5) it seems that the pattern of sodium excretion after sodium chloride load is dependent on the intrarenal arterial pressure and on the transport of solute from the renal medullary fluid by the vasa recta Therefore a normal or abnormal pattern of sodium excretion cannot be considered directly indicative of the absence or presence of a renal factor in hypertension, as suggested by Timmis and Gordon (11) As yet no conclusive evidence of a hyperfunction of the renal pressure mechanism in aortic coarctation has been presented (9 Admittedly, a severe constriction of the suprarenal aorta in experimental animals causes hypertension and appearance of

detectable renin in the blood of renal veins but, on the other hand a mild constriction corresponding in degree to that seen in human coarctation releases no detectable renin from the kidneys (10) In one studied case of aortic coarctation the granularity of the juxtaglomerular cells was normal (4) However, no better evidence of the absence of a renal pressure factor is available More sensitive methods of renin assay and better understanding of the details of renal blood pressure regulation may well dispel our present uncertainty as to the mechanism of hypertension in aortic coarctation

Other factors not studied in this work which might decrease natriuresis in aortic coarctation are decreased glomerular filtration rate or its decreased response to saline loading, different response of renal blood flow, increased aldosterone excretion, and direct effects of angiotensin on tubular reabsorption of sodium

Summary

Renal excretions of sodium potassium and total osmols, and changes in the serum sodium and potassium concentrations were studied after an intravenous infusion of hypertonic sodium chloride in eight adult subjects with aortic coarctation and in a control group of six subjects A significantly smaller renal excretion of sodium in the coarctation group was found the difference increasing with time No significant difference in the renal excretion of potassium and osmols or in the changes of the serum sodium and potassium concentrations was found between the coarctation and control groups Thus, the pattern of renal sodium excretion after sodium chloride load differs sharply from that observed in essential hypertension and corresponds to the normal to subnormal sodium excretion found in renal hypertension These results are consistent with the hypothesis that the pattern of renal sodium excretion is modified by the amount of blood flowing through the vasa recta which in turn is regulated by the intrarenal arterial pressure pulse pressure or pulsatile flow

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TABLE I. The material with number of individuals in different age groups, average and range values for age, height, weight and commencing age for physical training

No of individuals	Age years	Height (cm)	Weight (kg)	Beginning year for physical training (age years)
14	46	175.9	7[illegible].3	18
	42–49	168–183	66.0–83.3	11–2[illegible]
15	54	173.7	68.0	20
	50–59	168–18[illegible]	61.0–[illegible]8	13–35
4	6[illegible]	169.8	64	19
	61–68	157–1[illegible]9	58.0–[illegible]4.5	16–20

area the runner has to find his own way to 5–10 different spots such as a small hill, swamp etc. with the aid of a map and a compass. The training programme which the subjects regularly follow during at least 8 months of the year consists of at least 1–1.5 hours running 2–6 times a week. The runner varies his speed from running with a slow speed up to repeated 3–15 minutes intervals with top speed. Twelve subjects in this group also trained for and competed in cross-country skiing.

Out of a total of 33 subjects 16 came from the western part of Sweden and were studied in Göteborg, whereas 17 from the eastern part were studied in Stockholm. The investigation was performed during the ordinary competition season for orienteering.

Procedure and methods

Normally the subjects came to the laboratory on two different days. The first day a clinical examination including blood pressure measurements and ECG recording at rest was performed before the exercise tests (bicycle ergometer) on two submaximal loads and one maximal load were started. On the second day the subject was in a fasting state. Red cell volume, other blood analysis, dynamic spirometry and heart volume determination

TABLE II. Rest values. Means, standard deviation (except for the oldest group) and range are …

Age group years	No	Heart rate beats/min	Heart volume ml	Blood pressure (mm Hg) Systolic	Diastolic
42–49	14	5[illegible]	1 0[illegible]4	135	83
		10	11[illegible]	1[illegible]	9
		31–[illegible]0	8[illegible]0–1 200	115–15[illegible]	70–100
50–59	1[illegible]	5[illegible]	93[illegible]	137	81
		10	1[illegible]3	18	11
		3[illegible]–[illegible]8	6[illegible]0–1[illegible]0	115–1[illegible]0	60–9[illegible]
61–68	4	[illegible]0	830	139	8[illegible]
		[illegible]0–[illegible]7	6[illegible]0–9[illegible]0	125–155	75–[illegible]

were performed and the exercise test was repeated on one or two other submaximal loads and one maximal load If there was no "levelling off" (22) and the difference was too wide between the two determinations of the maximal oxygen uptake (> 0 2 l/min) a third day was used for determining maximal oxygen uptake

Arterial blood pressure was measured *indirectly after at least 20 minutes rest in* the supine position with the auscultatory method using a mercury manometer The diastolic pressure was regarded as coincident with the sudden change in character and the *fading of Korotkow s sound*

In 15 subjects the red cell volume was determined with the Cr^{51} method (15) Haemoglobin concentration in blood was determined spectrophotometrically after con version to *cyanmethaemoglobin and haemat*ocrit with a high speed centrifuge (12 000 r/min) with a correction for trapped plasma of 1 5 per cent (16) Cholesterol in serum was determined according to Cramer and Isaksson (13) or a modified Schoenheimer and Sperry method (34) and neutral fat according to Carlsson and Wadstrom (12)

Heart volume was determined roentgenographically in the prone position The X ray tube used for the Goteborg material was at an angle of 30 degrees with the horizontal plane (26) but for the Stockholm group at no angle (25) When comparing the data for the heart volumes in relation to the maximal oxygen uptake there was no difference in the two materials and the data are treated together in table II and fig 3

Forced vital capacity and maximal voluntary ventilation (free respiratory frequency) was determined in the sitting position with a Bernstein spirometer (10 20)

Expired air was collected in Douglas *bags and the volume measured in a spirom*eter or a dry gasmeter Gas samples were analysed with Scholander or modified Haldane apparatus

The ECG was recorded at rest on a direct *ink jet writing 4-channel electrocardiograph* (Minograph 42 B Elema Schonander Stockholm) with the following leads I II III aVR aVL aVF $CR_{1\ 2\ 4\ 5}$ During exercise the indifferent electrode was placed *on the forehead (CH leads 24)* ECG was also taken at rest immediately 1 and 4 minutes after exercise The ECG findings were classified according to the Minnesota code (11) as modified by I Åstrand (5) Heart rate was determined *from* the ECG recording

Lactic acid was determined in blood taken from a prewarmed fingertip according to the slightly modified Baker and Summerson s method (38) Samples were taken according to I Åstrand (4 p 14)

The maximal isometric muscle strength was measured with strain gauge apparatus (3)

given

Haemoglobin (conc g/100 ml)	Haematocrit (%)	Cholesterol (g/100 ml)	Neutral fat (mM/l)
13 4	41 0	222	0 85
0 9	2 4	29	1 07
12 1–14 6	38–46	177–273	0 45–1 67
13 7	41 0	251	0 95
0 7	2 1	30	0 81
12 6–14 9	38–45	191–284	0 82–1 56
13 4	41 2	286	1 10
12 5–14 3	39–43	241–334	0 96–1 22

TABLE I The material with number of individuals in different age groups, average and range values for age, height, weight and commencing age for physical training

No of individuals	Age (years)	Height (cm)	Weight (kg)	Beginning year for physical training (age, years)
14	46	175.9	72.3	18
	42–49	168–183	66.0–83.3	11–25
15	54	173.7	68.0	20
	50–59	168–186	61.0–75.8	13–35
4	65	169.8	64.7	19
	61–68	157–179	58.0–74.5	16–20

area the runner has to find his own way to 5—10 different spots such as a small hill, swamp etc with the aid of a map and a compass. The training programme which the subjects regularly follow during at least 8 months of the year consists of at least 1—1.5 hours running 2—6 times a week. The runner varies his speed from running with a slow speed up to repeated 3—15 minutes intervals with top speed. Twelve subjects in this group also trained for and competed in cross country skiing.

Out of a total of 33 subjects 16 came from the western part of Sweden and were studied in Göteborg whereas 17 from the eastern part were studied in Stockholm. The investigation was performed during the ordinary competition season for orienteering.

Procedure and methods

Normally the subjects came to the laboratory on two different days. The first day a clinical examination including blood pressure measurements and ECG recording at rest was performed before the exercise tests (bicycle ergometer) on two submaximal loads and one maximal load were started. On the second day the subject was in a fasting state. Red cell volume, other blood analysis, dynamic spirometry and heart volume determination

TABLE II Results at rest. Means, standard deviation (except for the oldest group) and range are

Age group (years)	Nos	Heart rate (beats/min)	Heart volume (ml)	Blood pressure (mm Hg) Systolic	Diastolic
42–49	14	52	1 047	135	83
		10	116	12	9
		31–70	840–1 200	115–155	70–100
50–59	15	57	937	137	81
		10	1 3	18	11
		39–78	670–1 220	115–170	60–95
61–68	4	60	830	138	80
		50–77	620–970	125–155	75–85

TABLE IV Electrocardiographic findings

Code		Incident of changes		
		Years 42–49 Nos 14	Years 50–59 Nos 15	Years 61–6 Nos 4
	ECG changes at rest			
I 2	Q in AVL 0 03–0 04 sec R 3 mm or more	0	1	0
IV 3	ST junction elevation 2 mm or more	6	2	0
VI 3	PR ≥ 0 25 sec	2	1	1
IX 3	Frequent ventricular ectopic beats (≥4/40)	1	2	0
X 2	T waves 12 mm or more	6	10	1
X 3	Notching of QRS in CR 1 2 and/or marked S wave in CR,	7	5	0
X 7	Occasional supraventricular ectopic beats	0	1	0
	ECG changes in exercise test			
IV 1	ST junction depression 1 mm or more and ST segment horizontal or downward sloping	0	1	1
IV 2	ST junction 0 6–0 9 mm and ST segment horizontal or downward sloping	2	3	0
IV 3	No ST junction depression as much as 0 5 mm but ST-segment downward sloping and reaching 0 5 mm or more below PR baseline	0	0	1
V 4	No ST junction depression as much as 0 5 mm but ST segment horizontal or downward sloping but not reaching 0 5 mm below PR baseline	0	2	0
5	ST junction depression 1 mm or more and ST-segment upward sloping	0	2	2
6	ST junction depression 0 6–0 9 mm and ST segment upward sloping	2	1	0
,	Negative T wave 1–5 mm	0	1	1
	Wenchebach heart block during the first minutes after exercise	0	2	0
9	Episodes of sinus arrest after exercise	1	0	0
	Frequent ventricular ectopic beats after exercise (≥4/40)	0	1	0
	Frequent supraventricular ectopic beats after exercise (≥4/40)	0	1	2
	Occasional ventricular ectopic beats during or after exercise	2	1	0
	Occasional supraventricular ectopic beats after exercise or periods of ectopic atrial rhythm	1	2	1

showed however, a definite y to increase with age The heart l lactic acid concentration at a given O_2 uptake tended also to be higher in the two older than in the youngest of the three age groups

TABLE V. Maximal oxygen uptake, ventilation volume, heart rate and blood lactic acid concentration. Means, standard deviation (except for the oldest group) and range are given

Age group (yrs)	Nos	Oxygen uptake STPD (l/min)	Ventilation volume BTPS (l/min)	Heart rate (beats/min)	Lactic acid conc (mM/l)
40–49	14	3.98	118.5	175	11.2
		0.37	21.2	13	2.1
		3.50–4.49	76.3–146.8	148–193	8.0–15.6
50–59	15	3.39	113.7	176	10.8
		0.56	18.8	15	1.4
		2.49–4.19	84.2–154.6	154–203	9.4–14.0
61–68	4	2.78	94.8	165	9.5
		2.41–2.9	86.6–112.6	149–178	8.8–10.2

The ECG (table IV) at rest was characterized by high R and T waves. The tallest R wave in the precordial leads was recorded in CR_4 or CR_5 and averaged 30 (range 16–56) mm. The greatest R + S amplitude was 39 (range 19–61) mm. The T waves were tallest in CR_2 or CR_4 and averaged in either of these leads 13 (range 4–25) mm. Twelve subjects had minor intraventricular conduction defects, usually with notching of QRS in CR_1 or CR_2.

None of the subjects complained of chest pain during or after the work test. Two of the 33 subjects (6 per cent) had ST depressions which were classified in group IV 1 and 6 of 33 (18 per cent) ST-T changes which were classified in groups IV 2 and IV 3. As seen in table IV the incidence of such changes tended to increase with age. There was also a fairly high frequency of ectopic beats. Three subjects had frequent ventricular ectopic beats at rest, but less or none during exercise. Only in one athlete was there more ectopic beats after than during work. Four other subjects had frequent supraventricular ectopic beats during the first minutes after exercise. In two subjects Wenchebach heart block occurred during the first minutes after work. One of them had a prolonged P-R time (0.25–0.26 sec) at rest before exercise and six months later Wenchebach heart block was also recorded at rest before work. In the other subject the impulse origin varied after exercise. Both these two subjects had an aerobic capacity above 4 l/min and belonged to the most well trained in the present group. In another subject some episodes of sinus arrest were noted after exercise and two subjects had periods of ectopic atrial rhythm after work. None of these subjects complained of arrhythmia.

The maximal values for oxygen uptake, heart rate and lactic acid concentration in blood are given in table V.

In fig. 1 the maximal oxygen uptake is related to age. As in the following three figures the subjects with ST-

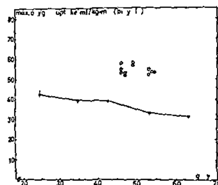


Fig 1 Individual values for maximal oxygen uptake in relation to age Filled circles are used for athletes with ST-changes classified as IV 1—2 (see table IV) The full line with ± 2 SD denotes a material of nonathletes of different ages (7) The unfilled rectangle denotes the mean value for the 9 best Swedish orientering runners of today (Saltin unpublished observations)

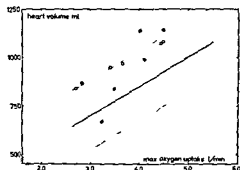


Fig 3 Individual values for heart volume in relation to maximal oxygen uptake Symbols see fig 1 The full line with ± 2 SD denotes a material of 32 healthy males age range 20—29 years (8 Saltin unpublished data) The equation for the regression line is y 257 + 149 9 x r = 0 81

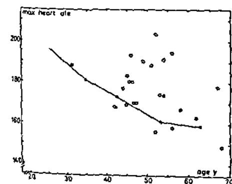


Fig 2 Individual values for maximal heart rate in relation to age Symbols see fig 1 The full line with ± 2 SD denotes a material of non athletes of different ages (4)

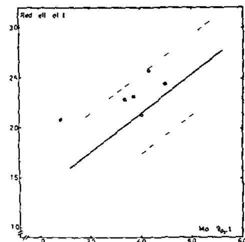


Fig 4 Individual values for red cell volume in relation to maximal oxygen uptake in 15 subjects Symbols see fig 1 The full line denotes the regression line ± 2 SD (y = 0 57 + 0 4 x r = 0 87) for 20 healthy males age range 20—40 years (Grimby Saltin Sanne and Soderholm unpublished data)

changes classified as IV 1—2 on ECG at exercise tests are marked with filled dots All athletes have maximal oxygen uptake above the means for the different age groups in earlier investigations (4 7)

The relationship between the maximal heart rate and age is showed in fig 2 There are large individual variations It can be mentioned that of two subjects of the same age one had the highest heart rate (203 beats/min) and the other had the lowest maximal rate (148 beats/min) They had about the same maximal oxygen uptake (4 10 and 3 81 l/min

TABLE V Maximal oxygen uptake ventilation volume heart rate and blood lactic acid concentration Means standard deviation (except for the oldest group) and range are given

Age group (yrs)	Nos	Oxygen uptake STPD (l/min)	Ventilation volume BTPS (l/min)	Heart rate (beats/min)	Lactic acid conc (mM/l)
42—49	14	3 98	118 5	175	11 2
		0 37	21 2	13	2 1
		3 50—4 49	76 3—146 8	148—193	8 0—15 6
50—59	15	3 39	113 7	176	10 8
		0 56	18 8	15	1 4
		2 42—4 19	84 2—154 6	154—203	9 4—14 0
61—68	4	2 68	94 8	165	9 5
		2 41—2 97	86 6—112 6	149—178	8 8—10 2

The ECG (table IV) at rest was characterized by high R and T-waves The tallest R wave in the precordial leads was recorded in CR_4 or CR_5 and averaged 30 (range 16-56) mm The greatest R + S amplitude was 39 (range 19—61) mm The T waves were tallest in CR_2 or CR_4 and averaged in either of these lead, 13 (range 4—25) mm Twelve subjects had minor intraventricular conduction defects usually with nothing of QRS in CR_2 or CR

None of the subjects complained of chest pain during or after the work test Two of the 33 subjects (6 per cent) had ST depressions, which were classified in group IV 1 and 6 of 33 (18 per cent) ST T changes which were classified in groups IV 2 and IV 3 As seen in table IV the incidence of such changes tended to increase with age There was also a fairly high frequency of ectopic beats Three subjects had frequent ventricular ectopic beats at rest, but less or none during exercise Only in one athlete was there more ectopic beats after than during work Four other subjects had frequent supraventricular ectopic beats during the first minutes after exercise In two subjects Wenchebach heart block occurred during the first minutes after work One of them had a prolonged P-R time (0 25—0 26 sec) at rest before exercise and six months later Wenchebach heart block was also recorded at rest before work In the other subject the impulse origin varied after exercise Both these two subjects had an aerobic capacity above 4 l/min and belonged to the most well trained in the present group In another subject some episodes of sinus arrest were noted after exercise and two subjects had periods of ectopic atrial rhythm after work None of these subjects complained of arrhythmia

The maximal values for oxygen uptake heart rate and lactic acid concentration in blood are given in table V

In fig 1 the maximal oxygen uptake is related to age As in the following three figures the subjects with ST-

The means standard deviation and range for the blood lactic acid are given in table III Fig 5 shows the lactic acid concentration in relation to the oxygen uptake as a percentage of the maximal oxygen uptake There was no difference between individuals of different ages in this relationship The steep increase of the lactic acid concentration occurred at a higher relative work load in the athletes than in a group of 56 sedentary living men aged 20—58 years (Grimby, Sanne and Soderholm unpublished results) In the athletes there was a marked increase in the blood lactic acid concentration at an oxygen uptake corresponding to about 80 per cent of the aerobic work capacity There was no significant difference in the maximal lactic acid concentration between the athletes and the control group or between the different age groups

The results from the dynamic spirometry are demonstrated in fig 6 The measured volumes are expressed as a percentage of the predicted normal value according to Berglund et al (10) and for the maximal voluntary ventilation according to Grimby and Soderholm (20) The vital capacity was normal or slightly increased except in two of the subjects in the oldest age group The forced expiratory volume in one second ($FEV_{1.0}$) and the maximum voluntary ventilation (MVV_F), however, were usually substantially higher than predicted

The leg extension for one leg averaged 78 (range 48—111) per cent of the predicted normal value (2) The average value for handgrip was 86 (range 76—97) per cent

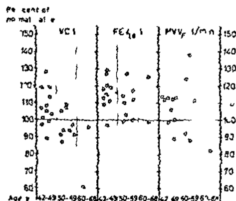


Fig 6 Vital capacity (VC) forced expiratory volume on one second ($FEV_{1.0}$) and maximum voluntary ventilation with a free frequency (MVV_F) as a percentage of predicted normal values (10 20)

Discussion

In this group of well trained middle aged and old men aerobic work capacities up to 4 5 l/min are found in the ages 42—49 years 4 2 l/min in the ages 50—59 years and 3 0 l/min in the ages 60—68 years The mean values for the different age groups are about 30 per cent above what is considered as average according to I Åstrand (4)

The red cell volumes and heart volumes are also large The red cell volume in our subjects fell within the relation to aerobic capacity found in younger men thus demonstrating the good correlation to the physical working capacity even in higher age groups (6 32) The haemoglobin concentration does not deviate significantly from values in the same ages given by Strandell (37) However as many as 10 men have haemoglobin values below 13 0 g per 100 ml

The heart volumes in about half the subjects are above 2 SD from the

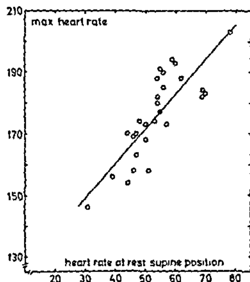


Fig 7 Individual values for maximal heart rate at exercise in relation to heart rate at rest in supine position The equation for the regression line is y = 115 + 1 1 x r = 0 81

regression line for aerobic capacity and heart volume calculated for younger men In former racing cyclists (41—66 years) heart volumes of similar size (23) as in the present material have been found, although the physical working capacity is larger in our material Individuals with a high aerobic capacity have unusually large heart volumes, which remains more or less unchanged when the maximal oxygen uptake de creases with age In the present material only one subject with abnormal ST changes during exercise shows a very large heart volume in relation to the maximal oxygen uptake but in the other subjects with ECG changes no such marked deviations are found This is in part in contrast to Strandell (36) who found an increase in heart volume with age and in his material there is a parallel increase in electro cardiographic deviations from the nor mal

The maximal heart rates fall roughly within the normal variation for the age (4) Low heart rates are usually found at rest, the lowest value being 31 beats/min There is a remarkably good correlation between the heart rate at rest and the maximal heart rate during exercise (r = 0 81, fig 7) Even if the heart rate at rest usually decreases during a training period (6), there is no correlation in this fairly homogenous material between the heart rate at rest and the aerobic work capacity At submaximal work intensities the heart rate is *considerably lower than that* seen in non athletic men (4, 19) The blood lactate concentration is considered as indicator for the degree of anaerobic metabolism In our control material of almost untrained subjects there is a steady increase in blood lactic acid concentration at work levels between 30 and 80 per cent of the aerobic capacity (fig 5) In the present group of athletes, however, the lactic acid concentration changes very little until about 80 per cent of the maximal oxygen uptake is used This might indicate a lower degree of anaerobic metabolism at a certain relative work load which might be one factor of importance for prolonged muscular per formance in athletes It is worth notice that the present group is not charac terized by a high maximal isometric muscle strength

As in previous reported materials of well trained men (33, 41) the ECG in the present group of athletes is characterized by tall R and T waves

as a result of the ventricular hypertrophy Minor intraventricular conduction defects usually corresponding to the right ventricle are also common

In the present material there is a fairly high frequency (24 per cent) of notable ST-depressions (IV 1—3) in the ECG at exercise Rumball and Acheson (30) found in men employed in the British Royal Air Force such changes in 14 per cent in the ages 45—49 years and 19 per cent in the ages 50—54 years In a material of 50—65 years old truck drivers I Åstrand (5) noted ST depressions of these types in 13 of 73 men (18 per cent) In another Swedish study of clinically healthy men (35) ST changes of similar type and classified as abnormal or suspected abnormal were found in 1 of 24 men in the ages 40—49 years 2 of 14 in the ages 50—59 years and 3 of 21 in the ages 60—69 years Thus compared with all these materials of non athletes in the same ages, there is a somewhat higher frequency of ST-depressions in the present material of well trained athletes In former racing cyclists ECG changes classified as pathological were found in 8 of 19 men but no detailed analysis is reported (23) The causes and the prognostic value of the observed ECG-changes are uncertain Long term studies will be of great value

There is a poor correlation between the presence of ectopic heart beats and ST changes which is confirmed in the present study Ectopic heart beats are quite common but not considerably more than in two Swedish control materials 5 35) It is remarkable that two of the most well trained athletes had Wenckebach heart block during the first minutes after exercise This might be an effect of an increased vagal tone (19), which also might explain the observed periods of ectopic atrial rhythm and sinus arrest

In a large normal group with varying physical activity, not including well trained athletes no correlation was found between heart rate at a submaximal work load and the results from dynamic spirometry (20) In the present group of athletes $FEV_{1.0}$ and MVV_F are considerably larger than predicted which to a certain extent could be due to muscular factors (1) The vital capacity shows no such obvious enlargement which is consistent with other reports (6) although larger vital capacities are found in well trained swimmers (9 31) The explanation may be that these athletes had performed either almost all their training before they had achieved full growth (9) or were under water swimmers (31)

The body build of these still active athletes is lean judged from skinfold measurements (Göteborg) and anthropometric data (Stockholm) The skin fold thickness in the subscapular region is within the range of 6 6—15 0 cm (mean 9 8) and in all cases below the average value in the population study by Tibblin et al (40, The fat free body weight determined from anthropometric data was on an average 60 kg (body weight 68 kg) The percentage fat is then 11 f per cent (range 5 0—16 1 which is in the same range as for trained young students (14)

Several investigations have shown that the serum cholesterol does not decrease during a relatively short train

ing period (21, 27) There is, though, the question whether extremely strenous exercise performed regularly during many years might give low cholesterol values The data from the present group which have trained regularly during at least 20 years, speaks against such a hypothesis The neutral fat level is, however somewhat low, which is consistent with the observations by Halloszy et al (21)

Characteristic of this group of well-trained middle aged and old athletes is the high aerobic work capacity, particularly if it is expressed per kilo of body weight Among the investigated links for the transport of oxygen, heart volume was markedly larger in relation to the maximal oxygen uptake compared with healthy young (see fig 3) and old males (Strandell, personal communication) In a part of the material haemodynamic studies have been performed, which show that the large heart maintains a stroke volume with a magnitude as great as expected when compared young males (18) The relatively high frequency of ECG changes was remarkable but no explanation was possible A follow up study is planned in order to evaluate the prognostic value of the ECG changes in this group of well trained individuals

Summary

1 Thirty three healthy males age range 42—68 years selected from a group of extremely well trained and still active long distance runners, have been studied regarding aerobic work capacity related dimensions, dynamic spirometry and by electrocardiogram recordings at rest, during and after exercise

2 Aerobic work capacity averaged for the age groups 42—49 years 3 98 l/min, 50—59 years 3 39 l/min and 61—68 years 2 68 l/min and in ml/kg × min 57, 53 and 43 respectively

3 Heart volume in relation to maximal oxygen uptake, compared with a group of healthy young males, was significantly larger in this group of still active middle aged and old athletes

4 There was no difference in the relationship of red cell volume to maximal oxygen uptake between the present group of athletes and younger controls

5 Dynamic spirometry showed larger $FEV_{1.0}$ and MVV_F than predicted

6 ST depressions were fairly common in the ECG after maximal exercise Among the arrhythmias noted there was Wenchebach heartblock in two athletes with intense training and high aerobic work capacity

7 Cholesterol in serum was not different from normal values in this age but neutral fat was lower than the normal mean values in most athletes The athletes had a lean body build

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A Genetic Approach to the Pathogenesis of Hepatic Cirrhosis

A Clinical and Serological Study

By

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t has been demonstrated that patients with predominantly non alcoholic cirrhosis of the liver can form antibodies to endogenous tissue substances Complement fixing antibodies to homogenates of liver tissue were demonstrated as early as 1908 by Fiessinger (10) and later by others (1, 6 11, 12, 23) By an immuno fluorescence technique it has been possible to demonstrate antibodies to cytoplasmic components in the epithelium of the biliary tract (24, 25) and to mitochondria (30) Immuno globulins capable of agglutinating latex particles sensitized with human γ globulin may often be demonstrated in the serum of patients with hepatic cirrhosis (2, 5) Special interest was attached to the finding of a positive L E cell phenomenon in a few of these patients (18 22) and to the demonstration of other anti nuclear globulins in the serum from almost one third of patients with hepatic cirrhosis (3 4, 8)

The role of these circulating tissue antibodies is unknown A direct pathogenic action has not been demonstrated Recent investigations indicate that the presence of some of these factors may be genetically conditioned For instance, the relatives of patients with systemic lupus erythematosus have exhibited an increased occurrence of antinuclear factors (9 15 21, 26, 27 32) and of hypergammaglobulinaemia (19) Similarly rheumatoid factors are more commonly present in the sera from relatives of patients with rheumatoid arthritis (20 33) than in sera from control subjects Patients with thyroid disorders have shown a familial predisposition to an increased occurrence of thyroid antibodies (13 28) and in the relatives of patients with pernicious anaemia antibody to parietal cells is more common than in control series (29) In the same families clinically manifest disease also appears to be more common than in the general population

The object of the present study was to elucidate whether similar genetic factors might be operative with respect

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to the occurrence of various tissue antibodies in patients with hepatic cirrhosis Sera from patients with hepatic cirrhosis and their full siblings were investigated for antinuclear factors rheumatoid factor, thyroid antibodies, and the concentration of γ globulin More over, a clinical study, with history taking, was made of the patients' relatives

Material and methods

The *proband series* comprises 38 patients with hepatic cirrhosis 24 females and 14 males In addition to the clinical and biochemical signs of chronic damage to the hepatic parenchyma the diagnosis was confirmed in all cases by liver biopsy or by autopsy In the histological diagnosis of hepatic cirrhosis the criteria were a disorganized architecture of the liver parenchyma and proliferation of connective tissue On the basis of the history the patients disease was classified as post hepatitic type (13 patients), alcoholic type (9 patients) or cryptogenic cirrhosis (14 patients) Two patients had biliary cirrhosis secondary to traumatic stricture of the biliary tract

The *relatives* were 90 full siblings of these 38 probands For practical reasons the investigation was restricted to siblings resident in Copenhagen and its closest environs This material comprises 53 females and 37 males ranging in age from 30 to 79 but with only 10 69 years All the siblings were interviewed personally according to a questionnaire which posed questions concerning not only their past and present diseases but also symptoms suggestive of lupus erythematosus rheumatoid arthritis hepatic renal and thyroid disorders and skin diseases atopic diseases and severe infections If an accurate diagnosis could not be confirmed the symptoms were recorded All the siblings had blood samples drawn for determination of antinuclear factor (ANF) rheumatoid factor (RF) and thyroid antibody while serum proteins were determined only in the sera from 80 siblings

The *control series* consists of the spouses of the studied siblings and 194 blood donors Owing to failure to attend or to lack of contact, this main control group comprises only 49 spouses and no attemp was made to supplement it in order to avoid selection This material comprises 25 females and 24 males ranging in age from 30 to 79 Five spouses were over 69 years old On being questioned, all the blood donors denied symptoms of collagenous diseases or a familial predisposition to such diseases and all had been in good health within the past 3 months This group comprises 65 females and 129 males ranging in age from 18 to 70

Gamma globulin was determined spectrophotometrically after paper electrophoretic fractionation

Antinuclear factor was determined by the immuno fluorescence technique described by Weller and Coon (31) For details cf Faber and Elling (8) As the nuclear source thyroid tissue from thyrotoxic patients was used A serum was considered positive when it gave undiluted fluorescence of the nuclei

Rheumatoid factor was determined with the aid of latex particles sensitized with human γ globulin (Hyland reagent) The reaction was considered positive if in a dilution 1/20 or higher in a glycine buffer a serum caused agglutination of the sensitized latex particles

Thyroid antibodies were determined by the above mentioned fluorescence technique With this technique a serum was considered positive if it gave undiluted a fluorescence of the cytoplasm or of the colloid in the thyroid section

Results

Probands As is evident from table I, ANF was found in the serum from half of the 38 probands, viz 16 females and 3 males High titres of ANF were found in 13 ANF was demonstrated in the sera from 11 out of 13 patients with

TABLE I Type of cirrhosis and serological findings in 38 probands

Type of cirrhosis	No of probands	ANF positive	RF positive	Cytoplasmic thyroid antibodies
Posthepatitic	13 (11 F—2 M)	11	8	2
Alcoholic	9 (2 F—7 M)	1	3	0
Cryptogenic	14 (10 F—4 M)	7	3	1
Biliary (secondary)	2 (1 F—1 M)	0	0	1

TABLE II Antinuclear and rheumatoid factor and thyroid antibodies in sera from 90 relatives of patients with hepatic cirrhosis and in two control groups

	Number		ANF positive		RF positive		Cytoplasmic thyroid antibodies	
Relatives		♀ 53		♀ 13		♀ 8		♀ 7
	90		20		15		9	
		♂ 37		♂ 7		♂ 7		♂ 2
Main control group		♀ 25		♀ 3		♀ 1		♀ 3
	49		3		4		5	
		♂ 24		♂ 0		♂ 3		♂ 2
Blood donors		♀ 65		♀ 5		♀ 8		♀ 3
	194		9		13		5	
		♂ 129		♂ 4		♂ 5		♂ 2

posthepatitic cirrhosis in the serum from one out of 9 patients with alcoholic cirrhosis and in the sera from 7 out of 14 patients with cryptogenic cirrhosis

The test for RF was positive in the sera from 14 out of 38 probands (37 %) most often in patients with posthepatitic cirrhosis (62 %) most rarely in patients with cryptogenic cirrhosis (21 %) In patients with alcoholic cirrhosis the reaction was positive in one third

Thyroid antibodies were demonstrated in only 4 out of the 38 probands

Relatives The results are shown in table II ANF was found in the sera from 20 out of 90 full siblings investigated From the table it may be seen that this reaction was also positive in the sera from 3 out of 49 spouses and from 9 out of 194 blood donors The ANF reaction was somewhat more common in females (female relatives 25 % female controls 6 % and female donors 8 %) than in sera from males male relatives 19 % male controls 0 % male donors 4 %) while there was no definite age difference

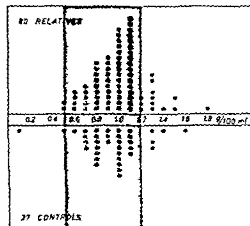


Fig 1 Distribution of gamma globulin values in relatives and controls

RF was found in sera from 15 out of 90 relatives (17 %), but also in the sera from 4 out of 49 spouses and from 7 % of the donors With advancing age there was an increasing incidence of positive reactions

Cytoplasmic thyroid antibodies were found in the sera from 10 % of the siblings as well as the controls by use of the immuno-fluorescence technique

The γ globulin concentration was determined in 117 persons 80 relatives and 37 controls Levels above 1 2 g/100 ml serum were found in 6 controls and in 15 relatives Levels above 1 6 g were found in 2 relatives, while no control had levels exceeding this limit (fig 1)

Table III gives the incidence of positive reactions among the relatives of seropositive (ANF and RF positive) probands It may be seen that ANF was demonstrated in the sera from 13 and RF from 9 out of a total of 42 relatives of probands exhibiting a positive ANF reaction This finding may be compared with a positive reaction in 2 out of 21 relatives of probands giving a negative reaction to ANF as well as to RF It may be seen also that a positive reaction for ANF and RF was found in 22 out of 42 relatives (52 %) of probands giving a positive ANF reaction

There was no definite correlation between the seropositivity among the relatives and the type of cirrhosis in the corresponding probands

Clinical findings

Hepatic diseases Data concerning previous jaundice were reported by 13 siblings and by 9 controls One sibling had histologically confirmed hepatic cirrhosis, while in 7 cases the history indicated that the previous jaundice was caused by acute hepatitis In the re

TABLE III The serological findings in 90 relatives compared with the occurrence of positive and negative ANF and RF in 38 probands with hepatic cirrhosis

	No of probands	No of relatives	No of relatives with positive reactions for		No of relatives with positive reactions for ANF and RF
			ANF	RF	
Sero-neg probands	11	21	2	2	4
ANF pos probands	19	42	13	9	22
RF pos. probands	14	35	8	8	16
ANF+RF pos probands	7	17	6	4	10

maining cases the most likely cause was gall stones

Articular diseases According to the history and clinical findings 5 relatives had evidence of rheumatoid arthritis No control subject had articular complaints

Thyroid disorders were demonstrated in 8 relatives and 2 controls Non toxic goitre was found in 4 relatives and 2 controls Three relatives had had operations for toxic goitre and one relative had myxoedema

Information concerning other diseases was given by only a very few siblings and spouses — and with the same frequency in the two groups In no case was there evidence of systemic lupus erythematosus

Discussion

All the probands had histologically confirmed hepatic cirrho is and make up a representative series as they do not differ — in respect of sex age, or the distribution of the various types of cirrhosis — from corresponding Scandinavian series (cf e g Hallen and Krook (17)) Signs of involvement of other organ systems were demonstrated in a few patients but actual lupoid symptoms or po itive L E cell pehnomena were not found Other tissue antibodies were, however, found in a large number of the probands Thus ANF was demonstrated in the serum from half the patients which is more than in other series (3 7) and RF was common in all the named types of hepatic cirrhosis Histologically there was fairly good agreement with the classification by history of the types of hepatic cirrhosis Most of the patients had a post necrotic hepatic cirrhosis or a fine granular nutritional cirrhosis, but seropositive as well as seronegative probands all showed varying degrees of lymphocytic infiltration in the liver

The clinical part of the present family study gave no evidence of familial predisposition to cirrhosis but it must be emphasized that no actual liver function tests were carried out In 7 cases there was a history of hepatitis, but these relatives had been in good health for many years, and none showed signs of recurrence or chronicity The frequency of previous hepatitis was not higher than in the general population but it was striking that in all 7 cases the respective probands gave a positive ANF reaction

According to Leonhardt (21) the relatives of patients with systemic lupus erythematosus show a broad spectrum of serological and biochemical abnormalities — in addition to uncharacteristic diseases progressing to actual lupoid syndromes However neither Leonhardt nor others have demonstrated an increased occurrence of hepatic diseases in family studies based on collagen diseases Thus, among 242 relatives of patients with systemic lupus erythematosus Leonhardt found only 3 with chronic hepatitis an incidence which is no higher than the incidence of cirrhosis in Sweden

In the present study signs of collagen diseases were found in 5 relatives all of probands giving positive serological reaction All had been suffering from rheumatoid arthritis for many years but only one showed a positive latex reaction for rheumatoid factor

In the serological part of the study thyroid antibodies as determined by the fluorescent antibody technique were no more common in sera from the relatives than in sera from controls despite the fact that thyroid diseases were rather commonly found in the siblings

With the present technique for demonstrating ANF, the frequency of the reaction has previously been established in groups of patients with well known diseases and in healthy persons (8) In accordance with previous investigations the ANF reaction was found to be positive in nearly all patients with systemic lupus erythematosus Most of the reactions were highly positive, but moderately strong to weak reactions were found in almost one third of these patients and in 9 of 194 blood donors Account being taken of this finding as well as of the sex and age distribution in the siblings and spouses, the incidence of ANF among the siblings was significantly higher ($p = 1\%$) than in the control series while the occurrence of a positive RAT among the relatives showed only a tendency to be increased Furthermore it was evident that the incidence of ANF was even higher among the siblings related to probands who exhibited a positive ANF reaction Unlike family studies on other collagen diseases (21) the present study showed no increase in the occurrence of hypergammaglobulinaemia among the relatives, and unlike the proband series the relatives did not manifest a correlation of the ANF with a simultaneous hypergammaglobulinaemia

The findings here reported are thus in accordance with similar serological investigations performed previously on the basis of probands suffering from systemic lupus erythematosus Pollak et al (27), for instance, found ANF in the sera from half of 50 relatives of patients with systemic lupus erythematosus, but in a larger, subsequent series the reaction was positive in only 37 % of full siblings (26) A corresponding significantly increased occurrence of ANF has also been demonstrated by others (9, 15, 21, 32), only Holman and Deicher (16) using the less sensitive complement fixing technique, did not find an increased occurrence of ANF

This investigation thus appears to indicate that genetic factors may play a pathogenetic role in liver cirrhosis The serological factors investigated in this study are hardly operative in isolation, let alone the direct cause of disease, but appear to be auto immune phenomena which might be genetically conditioned This seems to be exemplified by the lupoid syndrome which occurs spontaneously in mice of the NZB strain, in which all individuals develop a lupus like disease with circulating antinuclear globulins Hybrids resulting from a crossing with normal strains regularly develop manifest disease and serological abnormalities (14)

Whether these serological factors contribute to the development of chronic parenchymal liver damage is however, not known

Summary

In sera from 90 siblings of 38 probands with hepatic cirrhosis 22 % were found to have antinuclear factor and 17 % gave a positive latex reaction for

rheumatoid factor, while hypergammaglobulinaemia and thyroid antibodies were not more common among the relatives than in 2 control series consisting of spouses of the investigated relatives and 194 blood donors 52 % of the relatives of probands having a positive ANF reaction showed a positive reaction either for antinuclear factor or for rheumatoid factor

The study gave no evidence of a familial occurrence of liver cirrhosis

Antinuclear factor was demonstrated in the serum from half the probands and a positive latex test was found in one third The reactions were most often positive in patients with post hepatitic cirrhosis and in patients with hypergammaglobulinaemia No definite correlation was however, found between the incidence of these reactions in the series of relatives and the type of hepatic cirrhosis in the probands

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Serum Enzymes in Acute Tachycardia

By

INGVAR RUNDE and JON DALE

Determination of serum glutamic oxal acetic transaminase, SGOT is of great importance in confirming the diagnosis of myocardial infarction (1, 10, 13, 23) Elevated SGOT values are found during the acute phase of the disease in 96 9 to 99 % (2 4) of the patients Normal SGOT values therefore most probably exclude myocardial infarction Elevated SGOT values however are of limited diagnostic value in the absence of other pathological findings GOT is widely distributed in the organism (11, 23 26) and SGOT is therefore found elevated in a variety of pathological states, also in conditions that may be mistaken for myocardial infarction

It is well known that paroxysmal tachycardia often appears in the course of acute infarction Consequently it is of considerable theoretical and practical interest to know whether paroxysmal tachycardia without myocardial necrosis can cause SGOT elevation How often will this eventually occur? From which organs does the increased SGOT originate?

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During episodes of tachycardia the heart action may reach a critical frequency, causing the stroke volume to decrease and finally leading to reduction of the cardiac output per minute This leads to congestion not only of the lungs but also of the liver and other organs containing GOT In congestion of the liver with concomitant hypoxia centrilobular necrosis may develop This has been found in shock (7) in congestion of the liver caused by heart failure for example after myocardial infarction (4, 6, 8, 19), and in lung disease with arterial hypoxemia (14) In acute tachycardia pathological activity of SGOT and serum ornithine carbamyl tranferase SOCT has been found (16) Simultaneous pathological elevation of SGOT serum glutamic pyruvic trans aminase SGPT and SOCT is found in some conditions with acute congestion of the liver (19)

The present investigation has been performed in order to answer the question raised above SGOT SGPT SOCT, and serum lactic dehydrogenase,

TABLE I Distribution of various types of paroxysmal tachycardia number of patients episodes and mean ventricular rate

	No of patients	No of episodes	Mean ventr rate
Atrial tachycardia	6	6	182
Atrial flutter	6	9	154
Atrial fibrillation	12	13	162
Ventricular tachycardia	3	22	185
Total	27	50	173

SLDH, have been determined in a series of patients with acute tachycardia without infarction OCT is specific for the liver (20) while the 3 other enzymes are also located in the heart and other organs (11, 12, 23, 25, 27) Pathological elevation of SOCT therefore indicates affection of the liver, and determination of this enzyme will give information of such affection in acute tachycardia

Methods

SGOT and SGPT were determined according to the method of Reitman and Frankel (22) in Karmen units Normal upper values are 40 and 35 units respectively

SLDH was determined according to Wroblewski et al (24 27) in international units Normal upper value 350 units

SOCT was determined according to the microdiffusion method of Reichard (18 21) and nesslerisation according to Vanselow (9) Normal upper value 0 25 μM NH_3 developed per 0 5 ml serum

Material

The investigation was carried out on patients with paroxysmal tachycardia filling the following criteria 1 Electrocardiographical recording of the tachycardia 2 Ventricular rate exceeding 140 per minute for at least 30 minutes 3 Myocardial infarction was excluded on common criteria negative history no changes in the electrocardiogram normal sedimentation rate no leucocytosis or temperature elevation

Fifty episodes of acute tachycardia filled the criteria In all cases the activity of SGOT, SGPT and SLDH was determined on three successive days together with sedimentation rate white blood cell count and ECG readings SOCT was determined in the last 35 episodes The patients were examined clinically particularly for signs of circulatory disturbances In the urine Schlesinger s test for urobilin and Erlich s test for urobilinogen were performed

Results

Table I shows the distribution of the different types of tachycardia Repeated episodes in two patients explain the large number of episodes of ventricular tachycardia, and 2 of these 3 patients were referred to our department for synchronized direct current shock therapy

Table II shows the distribution of the pathological enzyme values The only case of atrial tachycardia followed by elevated SGOT and SLDH values was one of adrenalin intoxication Pathological SGOT values were found in 28 %, SGPT in 20 % and SLDH in 26 % The activity of SOCT was increased in 8 of 35 investigated episodes In all, 16 episodes caused elevated values of one or more enzymes

Table III shows the distribution of the maximum pathological values found after episodes of supraventricular tachycardia Moderate signs of liver damage was found at autopsy in two patients

TABLE II Number of episodes giving pathological serum enzyme values The last column shows the number giving elevated values of one or more enzymes SOCT was determined after 35 episodes the other enzymes after 50

		Number giving elevated values of				
	Total no	SGOT	SGPT	SLDH	SOCT	Totally
Atrial tachycardia	6	1	0	1	1	1
Atrial flutter	9	3	1	3	2	3
Atrial fibrillation	13	3	3	2	1	4
Ventricular tachycardia	22	7	6	6	4	8
Total	50	14	10	12	8	16

TABLE III Episodes of supraventricular tachycardia with maximal pathological values of each enzyme Normal value = N *Episodes with clinical signs of circulatory failure are marked +*

Patient				SGOT	SGPT	SLDH	SOCT	C failure
1	♀	90	Atrial fibrillation	N	N	444		–
2	♂	57	Atrial flutter	51	N	385		–
3	♂	51	Atrial fibrillation	61	160	N		–
4	♀	79	Atrial flutter	49	N	358	0 49	–
5	♀	57	Atrial fibrillation	58	57	N	N	
6	♂	54	Atrial tachycardia	58	N	520	0 37	+
7	♀	61	Atrial flutter	94	95	502	0 99	+
8	♀	56	Atrial fibrillation	100	83	354	0 56	–

TABLE IV Episodes of ventricular tachycardia with maximal pathological values

Patient			No of episodes	SGOT	SGPT	SLDH	SOCT	C failure
9	♂	70	4 and 5	51	49	440		–
10	♀	63	6	64	N	358		–
			1	109	48	352	0 87	–
			2 and 3	341	738	780	1 3	+
11	♂	68	4	N	55	N	N	
			3	41	N	N	0 33	–

who died in the hospital (patients nos 4 and 7 table III)

Table IV demonstrates the corresponding values after ventricular tachycardia All 3 patients had previously had myocardial infarction but normal SGOT values were recorded between the infarction and the first episode

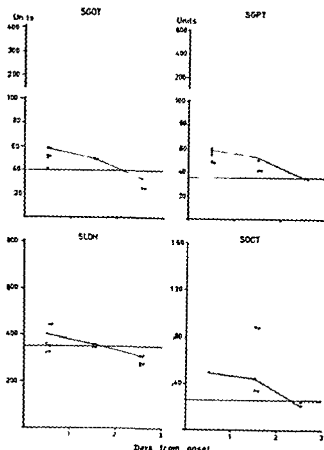


Fig 1 Enzyme values in relation to the onset of tachycardia Only episodes with one or more pathological values of the actual enzyme are included The mean values and upper normal limits are drawn

included in the material The first patient died later at home The third died in the hospital, and the liver appeared to be normal histologically Where two episodes occurred on two successive days it was difficult to know the contribution of each of them to the enzyme activities found These episodes are listed separately

Fig 1 shows the enzyme values in relation to the onset of tachycardia only episodes with one or more pathological values of the actual enzyme being included

In table V the SGOT values are related to heart rate and to the duration of the episodes As might be expected high ventricular rate and long duration of the episodes tend to cause SGOT elevation

In 19 episodes of tachycardia we found clinical signs of circulatory failure, based chiefly on the signs of pulmonary congestion Of the 14 episodes with elevated SGOT values 13 belonged to this group Regarding SOCT this enzyme was determined in 11 episodes followed by circulatory failure, and all 8 cases with elevated SOCT levels were found in this group

The mean value for the SGOT/SGPT ratio was 1 56 for patients with

TABLE V SGOT in relation to ventricular rate and to the duration of the episodes

	Ventricular rate				Duration of episodes	
	140—170	171—200	>200	Mean value	Less than 4 hrs	More than 4 hrs
SGOT elevated	4	9	1	186	5	9
SGOT normal	14	21	1	167	26	10
Total	18	30	2	173	31	19

TABLE VI Relation between SGOT and the pathological values of the other 3 enzymes

		Pathological values of		
	Total no	SGPT	SLDH	SOCT
SGOT elevated	16	9	11	8
SGOT normal	34	1	1	0

pathological SGOT, compared with 1 29 for patients with normal activity of this enzyme

The results of the tests for urinary urobilinogen (Erlich) and urobilin (Schlesinger) showed no significant difference between the two groups

We found good correlation between SOCT and the other enzyme values, especially between SOCT and SGOT (table VI) All 8 episodes followed by increased SOCT activity also gave pathological SGOT values Only in one case was the SGOT found to be moderately elevated (58 units) while the SOCT was normal

Discussion

With our criteria for the diagnosis of paroxysmal tachycardia without myocardial infarction we have found pathological SGOT values after 28 % of the episodes We have found a good correlation between the investigated enzymes especially between SGOT and SOCT In fig 1 the 4 curves are very similar both with regard to elevation and shape This suggests that the elevated levels of the different serum enzymes are caused by one and the same pathophysiological process Since OCT is located only in the liver it is probable that also the other 3 enzymes have had their main origin there The good correlation found between the pathological enzyme values and circulatory failure during tachycardia is interesting Passive congestion does not of course, occur only in the liver The possibility that SGOT SGPT and SLDH are also derived from organs other than the liver, cannot be quite excluded Nevertheless the results pre

sented strongly indicate that the increased serum enzyme activities are principally caused by liver cell damage

Regarding SGOT and SGPT, elevation is found in 14 and 10 cases respectively The SGOT/SGPT ratio in our material with demonstrated affection of the liver is higher than normal, in contrast to that previously described in liver disease (5 19, 25) However, there was considerable variation from case to case (range 0 46 to 2 64) As the enzyme activity is only moderately increased in most cases with pathological values, a small difference in elevation of one enzyme will cause a considerable change in the ratio The SGOT/SGPT ratio is therefore of little value in demonstrating liver affection in tachycardia

In many cases episodes of tachycardia are caused by acute myocardial infarction, giving elevated SGOT values Our investigation clearly demonstrates however, that tachycardia without myocardial necrosis is sometimes followed by elevated serum enzyme values SOCT-elevation strongly indicating liver cell damage In the presence of acute tachycardia increased serum enzyme activities therefore do not necessarily suggest simultaneous myocardial infarction

Summary

Three serum enzymes, SGOT SGPT and SLDH were determined after 50 episodes of tachycardia without myocardial infarction They were found elevated after 14, 10 and 12 episodes respectively SOCT was determined after the last 35 episodes, and pathological values occurred after 8 of them Circulatory failure was found in 19 episodes, including 13 with high SGOT values and all 8 with pathological SOCT Very good correlation was found between the 4 enzymes especially SGOT and SOCT, suggesting that the elevated serum enzyme values were caused by congestion of the liver, since OCT is specific for this organ The aid of SGOT and SGPT determination is limited in distinguishing between liver and heart affection, while SOCT is of great value in demonstrating liver cell damage

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Quinidine on Hemodynamics in Man

A Double Blind Study of Oral Treatment with Sustained release Tablets for a Week

By

G Schröder

Quinidine is used extensively in patients with cardiac rhythm disorders either as a short period treatment to convert paroxysmal arrhythmias or as a long term treatment to maintain normal sinus rhythm after conversion of atrial arrhythmias or to prevent ventricular beats The valuable depressive action on cardiac irritability that is therapeutically used however is accompanied by negative inotropic effect on the ventricular muscle In order to explore this action during continuous peroral treatment this study was performed in subjects without cardiac disease

Material

Four male and 2 female hospitalized patients took part in the study (table I) The reasons for hospitalization were suspected urinary tract disorder (L S), chest pain (K N), evaluation of a continuous extracardiac murmur (venous hum) (J A), peptic ulcer disease (S S), gallbladder disease (J B) and suspected cerebrovascular disease in one (E O) after a short time of left [illegible] Physical examination and ECG showed them to have normal circulation All except one (J B) who was obese had normal body build

Method

The patients agreed to take part in the study after having been carefully informed about the procedure and the aim of the study A double blind technique was used Four tablets were given twice a day The active tablet contains an amount of quinidine corresponding to 0 16 g sulfate in a sustained release skeleton (Duretter Hässle) Electrocardiographs with 11 leads were recorded before the study and at least twice during each treatment period of one week in order to be able to detect toxic symptoms

The details of the circulatory recordings have been reported (9) In the morning with the patient in a postabsorptive state polyethylene catheters were placed one in the brachial artery and one in the right atrium After intervals of half an hour sets of recordings including ECG cardiac output with a dye dilution technique intravascular pressures and expired air in a Douglas bag were performed first in the recumbent and

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TABLE I Anthropometric and treatment data of the material

Case	Sex	Age (yrs)	Height (cm)	Weight (kg)	Quinidine (no of days)	Placebo (no of days)	Placebo first	Remarks
L S	♀	32	166	66 0	5	6	—	0
K N	♂	36	184	77 2	7	6	+	Observed difference between the tablets
J A	♀	42	165	57 8	7	3	+	0
S S	♂	43	185	91 0	7	8	—	0
J B	♂	53	172	96 0	7	6	+	Nausea on quinidine slight
E O	♂	57	184	93 4	7	5	+	More tired after work on quinidine

then in the sitting position Thereafter they performed a piece of work sitting on a bicycle for 10 minutes at the end of which the same set of recordings was made

At the first study the patients had taken one kind of tablets for 3—7 days and at the repeated study the other kind of tablets for 6—8 days During the first treatment 4 patients were given placebo and 2 quinidine tablets

Mean values standard errors and P values using t test on individual differences were calculated The peripheral resistance units were calculated from the difference between pressure in the brachial artery and the right atrium *in mm Hg over cardiac* output in l/min An expression for the left ventricular work and stroke work was calculated using the brachial arterial pressure times the cardiac output and stroke volume respectively

Results

Three of the patients noticed a difference between placebo and quinidine tablets One (K N) had a feeling of stronger heart action and another (E O) became more tired during work while on quinidine Slight nausea appeared in one (J B) The remaining 3 noticed no difference Changes referable to quinidine were not seen on ECG

The hemodynamic data are presented in tables II and III

The heart rate during rest and exercise was unchanged during quinidine treatment No change in the reaction of the heart rate when changing from recumbency to sitting or when changing from rest in the sitting position to exercise on the bicycle was observed

The arterial pressures changed during quinidine treatment When changing position from lying to sitting the systolic pressure decreased on quinidine (8 0 mm Hg) while on placebo it increased (3 0 mm Hg) (P 0 05—0 025) This positional pressure reaction did not reach significant values for diastolic and mean pressures The pulse pressure

reaction however paralleled the systolic decrease The systolic pressure during exercise was lower on quinidine than on placebo 164 2 and 183 5 mm Hg respectively (P 0 05—0 025)

The systolic and mean pressure increase during exercise were less on quinidine treatment than on placebo (P 0 025—0 02 and 0 05—0 025 respectively) The pulse pressure reaction, however, was not significant (P 0 1—0 05)

Cardiac output was higher during rest in recumbency on quinidine than on placebo No other change of the cardiac output was evident on quinidine

Change of stroke volume did not parallel the change in output (P 0 10—0 05) The positional decrease of stroke volume when sitting up however was more marked on quinidine treatment (P 0 05—0 025) The stroke volume reaction during exercise was the same

The level and reaction of peripheral resistance during rest and exercise were unchanged on quinidine treatment

The level and reaction of right atrial mean pressure during rest was also unchanged on quinidine but during exercise this pressure was higher — 0 7 and + 2 2 mm Hg respectively (P 0 02—0 01) The pressure increase from rest to exercise however did not vary

The work of the left ventricle was higher at rest in recumbency on quinidine (P 0 02—0 01) but the reaction of sitting up and of exercise were not different

The stroke work of the left ventricle changed only when sitting up on quinidine treatment It decreased more than on placebo (P 0 02—0 01)

Oxygen consumption was the same on quinidine as on placebo The pulmonary ventilation was smaller in recumbency during quinidine (P 0 02—0 01 No other ventilatory change occurred)

Respiratory exchange rate was the same

The arterio venous oxygen difference decreased as the cardiac output increased during rest in recumbency on quinidine (P 0 05—0 025) No other change of this parameter on quinidine treatment was noted

Hematocrit was unchanged

Discussion

This method of study on the effect of drugs given for about a week has been used earlier (8, 9 10, 11) It is thought to give information about the pharmacological effects of drugs in the situation of their usual administration on patients for several days The reproducibility of hemodynamic studies repeated at similar intervals is found to be good (7)

The heart rate was unchanged during rest and exercise On spontaneously beating isolated hearts however quinidine has a depressive action on the frequency (6) This effect may have been opposed by the depressive effect in situ of vagal activity tending to increase the frequency (4) The concomitant adrenergic blocking action of quinidine (4) however speaks against this theory In normal subjects unchanged heart rate has also been reported from other studies (3 12)

The systolic pressure during exercise was lower on quinidine treatment This pressure reaction was not evident for the diastolic and mean pressures It may

TABLE II Mean values standard errors and P values of circulatory data in six patients given placebo HR = heart rate beats/min BA$_{S\ D\ M}$ = brachial arterial systolic (S) diastolic (D) and peripheral resistance units RA = right atrial mean pressure in mm Hg LVW and tory exchange rate Hct = hematocrit

	HR		BA$_S$		BA$_D$	
	P	Q	P	Q	P	Q
Recumbent						
	65 7	69 5	125 3	126 7	72 8	74 0
	4 6	3 4	7 0	4 6	4 1	8 5
Sitting						
	67 7	73 0	128 3	118 7	73 5	69 8
	4 0	4 3	7 2	5 2	4 7	4 1
Exercise sitting on a bicycle	149 0	150 2	183 5	164 2	85 2	79 3
	7 7	5 9	16 4	12 8	3 7	6 3
			0 05 – 0 025			

	LVW		LVSW		Oxygen consump ml/min STPD	
	P	Q	P	Q	P	Q
Recumbent	n = 5		n = 5		n = 5	
	7 78	9 98	125	146	278	268
	0 82	0 99	8	14	14	13
	0 02 – 0 01					
Sitting	8 03	7 82	119	107	280	274
	0 68	0 93	8	12	23	9
Exercise sitting on a bicycle	24 9	22 0	166	148	1 558	1 559
	2 9	2 0	11	14	100	86

reflect a decrease of contractility of the left ventricle more than a change of the autonomous adaptation of the vascular bed which should have tended to decrease the pressures more uniformly A clue pointing in the same direction is the mean arterial pressure reaction when changing from rest to exercise which increase was lower on quinidine treatment A decrease in contractility is well known from acute studies with quinidine (1)

During rest in the recumbent position the cardiac output was higher on quini

(P) and qu nid ne (Q) by mouth for about a week

mean (M) pressures n mm Hg CO = card ac output l/m n SV = stroke o un e n ml $\frac{BAM\ RA}{CO}$

LVSW left ventr cular work and stroke work in kgM/m n and gM/beats resp I R respira

BA$_M$		CO		SV		$\frac{BAM\ RA}{CO}$		RA	
P	Q	P	Q	P	Q	P	Q	I	Q
		n 5		n—5		n—5		5	
93 7	94 7	6 27	7 60	101 4	110 6	14 ?	12 6	8	3 1
5 6	4 7	0 40	0 55	2 4	6 6	1 0	1 ?	0	0 9
		0 0?5—0 0?		0 10—0 05					
								5	
95 ?	89 9	6 18	6 39	9? 0	88 0	15 9	14 5		0 9
5 8	6 1	0 29	0 49	3 6	6 2	1 0	1 5		1 ?
									0 05
122 2	108 7	14 9	15 0	99 8	100 7	8 35	7 ?3	0	
7 7	10 1	0 9	0 6	3 1	5 8	0 62	0 84	0	1 3
								0 0	0 01

Ventilation l/m n BTPS		R		Oxygen AV-d ff ml/l		Hct		Pulse pressure	
P	Q	P	Q	P	Q	P	Q	P	Q
n 5		n=5		n=5					
10 0	8 46	0 848	0 766	42 0	35 5	38 5	37 8	52 5	5? 7
0 94	0 72	0 073	0 035	1 6	3 6	1 3	0 8	4 7	3 2
0 02	0 01			0 05—0 025					
10 27	9 80	0 812	0 82?	45 5	44 0	38 7	38 3	54 8	48 8
1 40	1 47	0 051	0 069	3 4	2 7	1 3	0 9	5 3	2 1
44 3	41 9	0 923	0 935	105 8	104 7	43 0	42 3	98 3	84 8
3 7	5 1	0 010	0 016	0 3	6 0	1 0	1 0	14 1	11 2
								0 05—0 0?5	

dine This change occurred at the same venous filling pressure to the right ventricle A decrease of the total resistance is a possible explanation However the *calculated resistance change did not* reach s gnificant values in the present small series During rest in the recumbent position no change in output has been found during a study of quinid ne in normal subjects (3)

The decrease of stroke volume when *sitting up was more marked on* quinidine This may also be due to decrease in con tractility of the myocardium especially

TABLE III Changes due to positional and activity alterations during placebo (P) and quinidine (Q)

	HR		BAS		BAD	
	P	Q	P	Q	P	Q
From recumbency to sitting	+2 0	+3 5	+3 0	−8 0	+0 7	−4 2
			0 05–0 025			
From sitting at rest to exercise	+81 3	+87 2	+55 2	+45 5	+11 7	+9 5
			P 0 025–0 02			

	LVW		LVSW		Oxygen consump ml/min STPD	
	P	Q	P	Q	P	Q
From recumbency to sitting	0 08	−0 96	−7	−35	+5 0	+10 0
	(0 1	0 05)	0 005–0 001			
From sitting at rest to exercise	+16 9	+14 2	+47	+41	+1 278	+1 285

as the venous filling pressure tended to be higher on quinidine The stroke work decrease on quinidine was also more marked when sitting up but this change was not so evident for the left ventricular work This was however higher during rest in the lying position paralleling the cardiac output increase on quinidine

Any change of adaption to exercise of cardiac output stroke volume left ventricular work or stroke work was not evident on quinidine

The decrease of stroke volume and work found at rest in single dose studies of quinidine measured by indirect methods for cardiac output on subjects in sinus rhythm (12) thus could not be verified

The oxygen consumption at rest and during exercise was unchanged Thus the increased work of the heart at rest in the sitting position during quinidine treatment did not call for an increase in oxygen consumption The arteriovenous difference decreased as the cardiac out put increased

At rest in the recumbent position there was a 16 % decrease of pulmonary ventilation on quinidine In the sitting position and during exercise no change occurred Depre sive effect on the res piration of high doses of quinidine are known but small doses are found to give a stimulation of the nervous system Neither of these pharmacological effects explains the present result In studies no change has been found (12) The prolonged use of quinidine over a week may have changed the drive of the respiratory center

In isolated preparations quinidine depresses the oxydative processes by interfering with various enzymes The

treatments Mean values and P values of d fferences For abbreviat ons see table II

BAM		CO		SV		BAM RA / CO		RA	
P	Q	P	Q	P	Q	P	Q	P	Q
+1 5	−5 2	−0 28	−1 32	−8 2	−22 1	+1 8	+3 ?	8	+0
			(0 1−0 05)	0 05	0 075				
+27 0	+19 2	+8 7	+8 6	+7 8	+12 7	−7 6	3		3 1
	0 05−0 075								

Ventilation l/min BTPS		R		Oxygen AV d ff ml/l		HCI		P se pressure	
P	Q	P	Q	P	Q	P	Q	P	Q
−0 08	+1 16	−0 004	+0 01	+1 3	+10 0	+0 2	0 5	+2	−)
								0	0 01
+34 0	+32 1	+0 111	+0 113	+60 3	+60 0	+4 3	+4 0	43 5	3)
								(0 1	0 05

glucose and free fatty acid metabolism for energy supply is depressed (2 5) In the present study with small doses in the therapeutic range no changes of oxygen consumption or of the respiratory exchange rate indicated such effects

The changes found on normal circulation in man are small and give no reason to strengthen the indications before instituting quinidine treatment However the increased load on the left ventricle in recumbency at rest the change of orthostatic reaction and the changes during exercise may be unfavourable during prolonged treatment The feeling of stronger heart action sometimes observed may be due to an increased volume load as in one of the present subjects (K N) who had higher cardiac output and pulse rate with unchanged pressures on quinidine

Summary

Six circulatory healthy subjects were given placebo and quinidine each for a week Cardiac output heart rate intra arterial pressures right atrial pressure pulmonary ventilation oxygen consumption and respiratory exchange rate were determined at rest in recumbency and sitting and during work sitting on a bi cycle at the end of each treatment period

Cardiac output in recumbency in creased on quinidine treatment as well as the left ventricular work but the pulmonary ventilation decreased In the sitting position no changes were recorded The systolic pressure however when sitting up decreased as did the pulse pressure and the stroke volume on quinidine when compared with the reaction on placebo

During exercise on quinidine the systolic pressure and pulse pressure were lower and the right atrial pressure higher than on placebo The systolic and mean pressure increase during exercise was also lower

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The Effect of a Synthetic β-1-24Eikosatetra J ε Corticotropin (30'920-Ba Ciba) on Plasma Cor ids and the Urinary 17-KS and 17-OHCS

By

B A Lamberg L Strandstrom and S Pesonen

During the last decade a number of biologically active peptide chains corresponding to parts of the corticotropin molecule have been synthesised (7 10, 17 21) and most recently the β 1 24eikosatetrapeptide (13) with a biological activity corresponding to 106 units mg (22 23) A corticotropic effect has been observed in man with the 1 19peptide (9 17) and the 1 23peptide (3, 5, 10) A few studies have been carried out with the 1 24peptide (2, 6 8 11 14 16, 18 24 25) The object of the present study was to compare the effect of the synthetic β 1 24eikosatetrapeptide (30 920 Ba Ciba) with that of the natural hormone (Cortrophine Organon) as regards the urinary excretion of 17-ketosteroids (17 KS) and 17 hydroxy corticosteroids (17 OHCS) and on the plasma corticoids Preliminary reports have appeared (15 19) A comparison of the effect on some fractions of the 17 KS and the 17 OHCS was also made the results will appear separately

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Material and methods

Studies were carried out on 1 je Since the object was to compare he eff t of the two substances in the same person the cases were not specifically selected but in part they represent a series of cases with endocrine disease in which a corticotropin stimulation test was carried out (table I

Each of the compounds used was administered as an 8-hour intravenous infusion of 500 ml of 5 per cent glucose Cortrophine was given in doses of 25 old or 75 new units and the eikosatetrapeptide in doses of 0 25 mg The infusion was started at 8 a m

The test period comprised 6 days Infusions were given on days 2 and 6 whilst days 1 3 4 and 5 were regarded as control days The two compounds were administered alternately so that in half the cases the natural compound was given on day 2 and in the other half the synthetic substance Urine was collected on all 6 days for determination of the 17 KS (12) and the 17 OHCS (1) Plasma corticoids were determined (20) from blood samples withdrawn at 8 a m 12 noon and 4 p m on days 1 2 5 and 6

When assessing the response a common basal control level was used for both com

TABLE I The patients studied

Case no	Age	Sex	Diagnosis
1	53	♂	Malabsorption syndrome
2	46	♂	Traumatic primary hypogonadism postoperative hypothyroidism
3	16	♂	Pituitary dwarfism
4	31	♂	Idiopathic hypoparathyroidism pernicious anaemia
5	35	♂	Panhypopituitarism
6	27	♂	Coarctation of the aorta
7	58	♂	Arterial hypertension diabetes mellitus
8	36	♀	Turner s syndrome (XO)
9	18	♀	Malabsorption syndrome
10	34	♂	Arterial hypertension
11	44	♂	Arterial hypertension diabetes mellitus
12	38	♀	Idiopathic tetany hyperoestrogenism
13	30	♂	Arterial hypertension
14	30	♀	Cushing s syndrome (bilateral hyperplasia)
15	30	♀	Anomaly of papilla of the left eye
16	16	♀	Hirsutism

pounds For the urinary steroids the control level consisted of the mean obtained from the excretion during days 1 3 4 and 5 the plasma corticoid control level consisted of the mean of the 8 a m samples drawn on days 1 2 5 and 6

Statistical treatment of data was carried out with a logarithmic technique The basal level defined previously was analysed for individual variations and day to-day variations The ratio of increase as regards the urinary excretion of 17 KS and 17 OHCS and the plasma corticoids was compared for both substances In the analysis the order of infusion was also specifically taken into account One case was excluded from the analysis (case 5) owing to too large a deviation from the group in general

Results

The effect of 30 920 Ba and Cortrophine on the urinary *17 KS* and *17 OHCS* and the *plasma corticoids* is shown in tables II to IV and in figs 1 and 2 Owing to the varying endocrinological states of the patients the individual responses vary to some extent In the tables the cases have been listed according to the order of infusions Statistical analysis did not reveal any significant difference between the two compounds, however, even when the order of infusion was taken into account

Discussion

The excretion of both 17 KS and 17 OHCS *increased similarly after stimulation* with 30 920 Ba and the natural corticotropin and no significant difference between the two compounds could be detected, which is in accordance with the observations of others (8, 11, 16, 14, 18 24) The same holds true with regard to the effect on the plasma corticoids (8, 11, 14, 25) In the present study a maximal level of stimulation was used Walser and Koller (25) using various levels of 30 920 Ba, observed in a few cases, however, an apparently maximal stimulation with 0 06 mg as regards the plasma corticoids, whereas the urinary excretion of 17 OHCS increased log linearly at least up to a dose of 0 25 mg With a dose of 0 125 mg of 30 920 Ba Montervino et al (18) observed no further increase in the plasma corticoids from three to six hours during a six hr infusion, in contrast to the response elicited by 12 5 units of the natural corticotropin, when the double dose of the compounds was used no difference in response occurred however This may have some bearing on the observation (25) that 0 125 mg

TABLE II Effect of 30 920 Ba and Cortrophine on the urinary excretion of 17 hydroxycorticosteroids

Case no	Basal excretion (mg/d)	After Cortrophine (mg/d)	(ratio)	After 30 920-Ba (mg/d)	(ratio)
1	9 3	28 0	3 00	31 1	3 34
3	9 2	24 2	2 62	21 8	2 37
7	16 2	42 9	2 64	78 9	4 86
8	7 0	14 5	2 07	31 1	4 45
9	11 3	45 2	4 00	41 8	3 71
11	20 3	57 6	2 84	72 8	3 60
12	13 5	44 5	3 30	20 4	1 51
15	13 3	31 1	2 34	54 5	4 10
Mean ratio			2 85		3 49
2	9 5	32 1	3 38	28 5	3 00
4	12 4	36 4	2 94	25 0	2 02
5	4 3	11 9	2 76	9 3	2 16
6	19 5	43 2	2 22	50 5	2 60
10	18 2	48 2	2 65	36 4	2 00
13	20 2	33 6	1 66	39 4	1 95
14	73 5	128 1	1 75	107 9	1 47
16	12 5	27 5	2 20	38 1	2 97
Mean ratio			[1]2 40		[1]2 28

Cases 1 3 7 8 9 11 12 and 15 were given Cortrophine in the first infusion and 30 920-Ba in the second In the other cases the order of infusion was reversed

[1] Case 5 excepted

of 30 920 Ba brought about a larger increase in the urinary excretion of 17-OHCS than did the natural corticotropin during the first eight hrs, whereas during the next two eight hour periods the excretion pattern was reversed This would indicate that the effect of the natural hormone is more protracted than that of the synthetic substance Also the effect of the natural hormone when given in an intramuscular injection is more prolonged than that of 30 920 Ba (16) Landon et al (16) observed no difference between the two substances as regards the plasma corticoids during a five hour infusion test A maximal stimulation was brought about by the infusion of 4 μg hr of the eikosatetrapeptide corresponding to a total of 0 020 mg

It seems evident that the practical importance of a substance like 30 920 Ba lies in the fact that owing to lower antigenicity the possibility of hypersensitivity reactions is profoundly reduced So far clinical trials on this particular point are very few but some patients showing hypersensitivity towards the natural hormone preparations have been treated with 30 920 Ba without any untoward effects (6 11 14) A similar difference in response as regards hyper

TABLE III Effect of 30 920 Ba and Cortrophine on the urinary excretion of 17 ketosteroids

Case no	Basal excretion (mg/d)	After Cortrophine (mg/d)	After Cortrophine (ratio)	After 30 920-Ba (mg/d)	After 30 920-Ba (ratio)
1	5 7	7 6	1 49	8 6	1 51
3	5 9	7 9	1 34	6 7	1 14
7	6 3	11 7	1 86	11 5	1 83
8	3 2	5 9	1 84	4 5	1 40
9	4 7	7 2	1 53	5 8	1 23
11	12 0	19 2	1 60	20 6	1 72
12	6 6	12 1	1 84	8 5	1 29
15	10 0	20 9	2 09	21 1	2 11
Mean ratio			1 70		1 53
2	5 9	9 7	1 64	11 1	1 88
4	12 0	15 7	1 31	15 6	1 30
5	1 6	1 8	1 13	2 2	1 37
6	27 8	57 3	2 06	62 8	2 26
10	9 8	16 9	1 72	14 1	1 44
13	13 5	18 4	1 36	18 6	1 38
14	23 8	29 5	1 24	27 9	1 17
16	8 1	12 5	1 55	11 7	1 45
Mean ratio			[1]1 55		[1]1 55

For explanation see table II
[1] Case 5 excepted

sensitivity skin tests in otherwise hyper sensitive subjects has been observed (4) This point is also, of course, of importance when ACTH stimulation tests are performed on subjects who may be assumed to have impaired adrenal function and in whom hypersensitivity reactions may prove fatal A short-term 30 920 Ba stimulation test where the plasma corticoids are measured 30 min after a single intramuscular injection has, indeed been suggested (26) for these reasons

Summary

The effect of a synthetic β^{1-24}eikosatetrapeptide corticotropin (30 920 Ba Ciba) on the urinary excretion of 17 ketosteroids and 17 hydroxycorticosteroids and on the plasma corticoids was compared with that of a natural hormone preparation in 16 subjects who were either endocrinologically normal or presented various endocrine disorders Maximal stimulating doses, i e 0 25 mg of the synthetic and 25 old (or 75 new) units of the natural substance, were used The preparations were administered in an eight hour intravenous infusion Plasma corticoids were measured four and eight hrs after the start of the infusion No statistically significant differences could be observed between the effects of the two compounds

TABLE IV Effect of 30 920 Ba and Cortroph ne on the plasma cort co ds

Case no	Rest ng conc (μg %)	After Cortroph ne 4 hrs (μg %)	(rat o)	8 hrs (μg %)	(rat o)	After 30 920 Ba 4 hrs (μg %)	(rat o	8 hrs g	rat o
1	—	—		—	—	—			—
3	12 9	29 9	2 32	34 3	2 66	29 4	2 28	35 8	2
7	14 7	41 5	2 83	43 8	2 98	36 8	2 50	40 0	[illegible]
8	16 0	49 5	3 09	59 5	3 72	55 0	3 44	4 0	4 61
9	15 6	35 1	2 25	36 2	2 32	37 7	2 4[illegible]	41 5	6
11	2[illegible] 4	56 0	2 50	69 0	3 08	59 5	2 65		
1[illegible]	14 2	46 0	3 4[illegible]	50 0	3 53	34 5	2 43	4[illegible] 5	3 28
15	16 6	—	—	44 5	[illegible] 68	40 1	2 42	[illegible]0	2 95
Mean rat o			2 74		3 00		2 59		3 1
2	16 7	44 8	2 68	52 8	3 16	38 2	2 29	50 8	3 04
4	11 8	32 1	2 72	40 4	3 42	31 3	2 65	36	3 06
5	1 6	20 7	12 90	26 5	16 50	16 4	10 20	18 9	11 8
6	23 6	51 0	2 16	57 3	2 4[illegible]	51 7	2 19	60 9	[illegible] 5
10	15 6	40 3	2 59	47 0	3 02	37 3	2 39	43 3	2 78
13	11 7	36 7	3 14	44 8	3 84	41 5	3 55	54 0	4 61
14	40 0	110 0	2 75	129 5	3 24	116 5	2 92	121 0	3 03
16	19 9	46 0	2 30	60 0	3 02	54 0	2 [illegible]0	—	—
Mean ra o			[1]2 6[illegible]		3 16		2 67		3 18

For explanat on see table II

[1] Case 5 excepted

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TABLE V. Clinical data in patients with liver disorders

Type of liver disorder[1] Age Sex	Morphology[2]	Bromsulphalein retention	Plasma proteins (g/100 ml plasma)	Albumin (g/100 ml serum)
1 Posthepatitic cirrhosis—severe 44 male	At operation liver cirrhotic and atrophic	Abnormal	6.5	3.8
2 Posthepatitic cirrhosis—severe 26 female	—	Abnormal	5.6	2.1
3 Alcoholic cirrhosis—severe 56 female	Laennec cirrhosis + massive fatty infiltration	Abnormal	6.0	2.3
4 Alcoholic cirrhosis—mild 44 male	Laennec-cirrhosis of moderate degree	Normal	6.5	3.9
5 Fatty liver 73 male	Fatty infiltration	Slightly abnormal	7.9	4.1
6 Posthepatitic cirrhosis—severe 42 female	Laennec cirrhosis and atrophy	Abnormal	6.8	3.0
7 Posthepatitic cirrhosis—severe 71 male	Laennec-cirrhosis with slight fatty infiltration	Abnormal	6.7	3.8
8 Alcoholic cirrhosis—severe 52 male	Laennec cirrhosis with slight fatty infiltration	Abnormal	6.6	3.8

[1] As severe were grouped cases of liver cirrhosis with jaundice or signs of portal hypertension such

[2] Findings at operation or by means of biopsy.

All subjects were given Lugol's solution (10 drops 3 times daily) from three days before the start of the study.

Results

Plasma radioactivity curves. A typical curve for the disappearance of the labelled ^{131}I-fibrinogen in normal subjects is given in fig. 1.

The volume of distribution was found to be between 3,100 ml and 3,400 ml for five male normal subjects in 15 minutes.

In the decay curve the plasma figures have been expressed as a percentage of the values at 15 min. after injection. No correction to zero time has been done. The decay of radioactivity from the seventh day and onwards appears for practical purposes to be linear in the logarithmic plot. This type of curve was seen in all cases studied. The retained dose (administered dose minus urinary excretion) as well as the extravascular radioactive pool (retained dose minus

Gamma globulin (g/100 ml serum)	GOT (glutamic oxalacetic transaminase) (units)	GPT (glutamic puruvic transaminase) (units)	Bilirubin (mg/100 ml plasma)	Alkaline phosphatase (units)	Relevant drugs given during study
1 4	177	180	1 4	21	Prednisolone for two days
2 2	106	47	1 8	11	Aldactone Chlorthalidone
2 2	193	131	3 1	15	Aldactone Betamethasone
0 9	31	31	0 7	6	—
1 5	33	33	—	3	—
2 0	30	14	1 6	2 4	Aldactone Diurgin
1 4	31	20	1 2	4	Dihydrochlorothiazide
1 4	28	16	1 0	9	Dihydrochlorothiazide

as ascites splenomegaly or oesophageal varices

plasma activity) has also been plotted in the figure

Excretion of ^{131}Iodine in urine The cumulative urinary excretion of ^{131}Iodine in 6 subjects, followed during 18 to 25 days, was found to be 82 per cent (range 71—89 per cent) of the dose injected In four subjects followed during 9 to 12 days a slightly lower excretion was noted The excretion in two normal subjects is shown in figs 1 and 2 and in one patient in fig 3

Plasma half life of ^{131}I fibrinogen

Control subjects The calculated values for T1/2 are given in table III The mean value for men and women was 4 7 and 4 4 days respectively No significant difference in half life was found between the two sexes nor could any influence of age be observed No significant fibrinolysis could be demonstrated during the study The fibrinogen values were within the normal range and remained constant during the study

Coagulation factors deficiency states The results of these investigations are listed in table IV Two cases were afflicted with

TABLE VI Half life of ^{131}I fibrinogen and coagulation factors in patients with liver disease

Diagnosis Age Sex	T½ (days)	Coagulation time in glass tube (min)	Coagulation time in plastic tube (min)	Bleeding time (min sec)	Recalcification time (sec)
1 Cirrhosis post hepatitis—severe 44 male	4 0	9	30	4 29	232
2 Cirrhosis post hepatitis—severe 26 female	3 6	12	12	–	206
3 Alcoholic cirrhosis—severe 55 female	2 2	8	18	8	205
4 Alcoholic cirrhosis—mild 44 male	[illegible]	12	21	–	231
5 Fatty liver 73 male	3 9	11	27	–	202
6 Posthepatitic cirrhosis—severe 42 female	5 8	5	–	1 45	121
7 Posthepatitic cirrhosis—severe 71 male	4 4	9	20	–	254
8 Alcoholic cirrhosis—severe 52 male	7 0	7	16	4 25	272
Normal values (22)		6–10	20 30	4 6	150 250

hemophilia A one with von Willebrand s disease one with *hypoproconvertinemia* and one with hypofibrinogenemia For the patient with hypofibrinogenemia the half life was slightly below the normal limit The other patients in this group showed normal values No significant fibrinolysis could be demonstrated and the fibrinogen values remained fairly constant during the study (table XI)

Liver disorders Nine patients with liver disease were studied of whom eight had cirrhosis and one fatty liver In one patient (case 3) the half life was markedly reduced and in three others (*cases 2 4 and 5*) it was slightly reduced The clinical data are given in table V and the turnover and coagulation studies in table VI In the values before the study two cases had elevated fibrinogen levels and the remaining normal values During the study case 2 had subnormal values (table XI) 4 of 7 cases were low in factor IX Low values for platelet counts were found in all patients but one All cases but two had increased factor VIII activity This was especially pronounced in cases 2 3 4 and 6 In four of the patients a moderately

Factor VIII (AHF) (% of normal)	Factor IX (% of normal)	Factor V (% of normal)	Factor II + factor VII (% of normal)	Fibrinogen (g/100 ml plasma)	Fibrinolytic activity (µg/ml/hr) pH 6.3 pH 7.1	Platelets (/mm³ × 10³)
235	53	84	62	0 33	0 0	136
740	65	33	33	0 30	130 127	60
926	50	75	36	0 27	40 0	85
305	99	145	125	0 55	0 294	400
[illegible]30	173	88	105	0 52	0 0	189
660	62	165	41	0 26	120 0	159
163	135	56	46	0 28	0 0	49
128	148	70	51	0 21	0 0	158
165–135	60–160	80–120	85–110	0 26±0 06	0–100 0–100	200–400

increased fibrinolytic activity was demonstrated. All patients were in steady state with respect to fibrinogen level (table XI).

Not included in the above mentioned series is a patient who during the study developed a hypofibrinogenemic state with rapidly declining radioactivity and fibrinogen levels in plasma. This patient showed further more strong fibrinolytic activity. The data of this patient are shown in table X. This case had the shortest half life found in this study being only 1.9 days. No determinations of fibrinogenolytic activity were performed.

Polycythemia vera. Six patients were studied. The clinical data are summarized in table VII and the turnover and coagulation studies in table VIII. Cases 1, 3 and 5 had half lives within the normal range whereas three other cases had lower figures. The coagulation analyses showed minor inconsistent variations from the normal range. The platelet number was markedly increased in cases 1 and 3 and slightly so in case 2. No significant fibrinolysis could be demonstrated and the fibrinogen values remained fairly constant during the study (table XI). The two patients with significantly elevated platelet

TABLE VII Half life of ^{131}I fibrinogen and clinical data in patients with polycythemia vera

Diagnosis Age Sex	T/½ (days)	Hb (%)	White cell count (/mm³)	Platelets (/mm³ × 10⁻³)	Sedimentation rate (mm/hr)	Size of spleen	³²P treatment
1 P v with massive thrombocytosis 50 female	4 4	82	21 500	4 600	3	Spleenectomized in 1955	—
2 P v in active stage 55 female	3 6	122	10 400	580	0	Not enlarged	—
3 P v with massive thrombocytosis 77 female	4 3	112	13 300	3 000	1	Not enlarged	—
4 P v in myelofibrotic stage 60 female	2 2	72	33 200	390	36	Below umbilicus	—
5 P v in active stage 51 female	3 8	112	4 000	270	0	Not enlarged	1957 1959 1960
6 P v in myelofibrotic stage 56 male	2 9	73	2 500	140	—	Enlarged to umbilical level	1960 1060 1961

number showed normal values for the half life, while the remaining four had borderline or significantly shortened half lives

Epidermolysis bullosa Two patients aged 11 and 20 with epidermolysis bullosa hereditaria were also investigated In both cases the half life was within normal limits The coagulation analyses showed normal values (table IX) No significant fibrinolysis could be demonstrated and the fibrinogen values remained fairly constant during the study (table XI)

Discussion

As has been shown by McFarlane fibrinogen may easily be labelled with ^{131}I without serious damage to the molecule (19) McFarlane points out in his study on the turnover of rabbit fibrinogen that in this protein not more than 0 5 atoms of iodine can be incorporated into the fibrinogen molecule if denaturation is to be avoided Denaturation was defined by McFarlane as a rapid initial catabolism of injected labelled protein In our study on human fibrinogen we have used fibrinogen preparations labelled with between 0 8 and 2 7 iodine atoms per mole No significant difference with regard to the extrapolated values for the plasma pool of fibrinogen was observed for the different preparations indicating that no denaturation had occurred The discrepancy regarding the results on human and rabbit fibrinogen might be ex

plained by the difference in structure of the two proteins It is known that rabbit fibrinogen in contrast to human fibrinogen in its fibrinopeptide part contains a tyrosine O sulphate residue located close to the bond split by thrombin during the coagulation process Iodination of this residue might give rise to a molecular species liable to rapid excretion

The specific radioactivity (table I) of fibrin in our study was only a few per cent lower than in the corresponding fibrinogen (fibrinopeptides being accounted for) These data suggest that only an unsignificant degree of denaturation had taken place during iodination The average extrapolation value for the plasma pools was 66 % which value is somewhat lower than that reported for rabbit fibrinogen by McFarlane (20) However, this could as well be explained by species differences as by denaturation

The half life of fibrinogen from several species has earlier been studied by using different isotopes for labelling as well as by administration of normal fibrinogen in afibrinogenemic states (table XII) For human and rabbit fibrinogen the half life values obtained with different isotope methods agree fairly well

The rapid turnover of fibrinogen as compared with other plasma proteins could be explained if this protein was rapidly consumed by means of a continuous coagulation and fibrinolysis taking place within the blood stream However turnover studies of fibrinogen in patients with coagulation factor deficiency states have shown that the turnover in these states is as rapid as in normal subjects (14 1, 24) In our study also, no *significant difference in half life value* was observed in patients afflicted with such defects This suggests that the rapid turnover rate of fibrinogen can only be accounted for to a small degree by consumption through intravascular fibrin formation Rausen et al in their study arrived at the same conclusion (24)

It is interesting to note that 3 out of 6 patients with polycythemia vera showed significantly increased turnover rates of fibrinogen In all six cases the marrow was crowded with hypertrophic megakaryocytes indicating an intense production of platelets It is of considerable interest that only those patients with normal platelet counts in the peripheral blood showed short half lives This may be explained by an increased peripheral destruction of platelets leading to thromboplastic activity, initiating a hypercoagulable state The latter again could explain a rapid consumption of fibrinogen Christensen also found a shortening of the half life of fibrinogen in one case with polycythemia vera (17) The latter author points out that this finding is in agreement with the well known tendency to thrombosis in these patients

It is remarkable that in four patients with cirrhosis of the liver an increased turnover rate for fibrinogen was noted *The rapid turnover in the patients with* cirrhosis of the liver as well as those with polycythemia vera could not easily be ascribed to an increased fibrinolytic or fibrinogenolytic activity as no increased plasma fibrinolytic activity was found Neither can short half life in the cirrhotic patients be explained by a generalized increased catabolic activity as turnover studies of gamma globulin β_1 lipopro

TABLE VIII Half life of ¹³¹I fibrinogen and coagulation factors in polycythemia vera

Diagnosis Age	Sex	T½ (days)	Coagulation time in glass tube (min)	Coagulation time in plastic tube (min)	Bleeding time (min sec)	Recalcification time (sec)
1 P. v. with massive thrombocytosis 50	female	4.4	9	18	–	210
2 P. v. in active stage 55	female	3.6	9	39	2 20	216
3 P. v. with massive thrombocytosis 77	female	4.3	8	25	1 25	212
4 P. v. in myelofibrotic stage 60	female	2.3	11	24	1 50	206
5 P. v. in active stage 51	female	3.8	14	40	3 30	177
6 P. v. in myelofibrotic stage 56	male	2.9	12	43	9	194
Normal values (22)			6–10	20–30	4–6	150–250

TABLE IX Half life of ¹³¹I fibrinogen and coagulation analyses in two patients with epidermolysis

Patient Age	Sex	T½ (days)	Coagulation time in glass tube (min)	Coagulation time in plastic tube (min)	Bleeding time (min sec)	Recalcification time (sec)	Factor VIII (AHF) (% of normal)
11	male	4.8	6	25	1 55	240	128
20	female	4.4	6	29	4 25	217	88
Normal values (22)			6–10	20–30		150–250	65–135

tein, and albumin (29, 30) in cirrhotic patients have shown normal or prolonged figures for the half life. The findings in some of the patients with liver cirrhosis and polycythemia vera are suggestive of an increased consumption by means of coagulation. This hypothesis is further strengthened by clinical and

Factor VIII (AHF) (% of normal)	Factor IX (% of normal)	Factor V (% of normal)	One stage prothrombin time (patient/normal)	Factor II + factor VII (% of normal)	Fibrinogen (g/100 ml plasma)	Fibrinolytic activity (μg/ml hr) pH 6 3/pH	Platelets (mm / 10)
48	185	93	17 0/15 5	88	0 32	0 0	4 0
74	—	74	17 0/17 3	75	0 28	0	
68	128	73	18 0/17 3	115	0 35	0 0	0
126	113	123	18 1/17 3	87	0 31	34 0	
115	72	125	16 0/14 5	88	0 29	0 0	
145	54	57	22 5/17 5	63	0 42	0 183	
65–135	60–160	80–120		85–110	0 26 ± 0 06	0 10 0 100	

bullosa hereditaria

Factor IX (% of normal)	Factor V (% of normal)	One stage prothrombin time (patient/normal)	Factor II + factor VII (% of normal)	Fibrinogen (g/100 ml plasma)	Fibrinolytic activity (μg/ml hr) pH 6 3/pH 7 1	Platelets (mm 10
140	105	16 0/17 0	87	0 27	114	231
66	133	17 0/17 0	76	0 30	70	218
60–160	80 120		85 110	0 26 ± 0 06	0–100 0–100	200 400

laboratory observations made in liver cirrhosis (4 16 31) It can however not be ruled out that the increased turnover in the cirrhotic cases could be due to treatment with different drugs which may influence coagulation e g corticosteroids Thus the patients showing short half lives of fibrinogen were

TABLE X Data on a patient with liver cirrhosis developing a hypofibrinogenemic state during the study

A $T^1/_2$ and coagulation factors in patient E.S

$T^1/_2$ (days)	Coagulation time in glass tube (min)	Coagulation time in plastic tube (min)	Bleeding time (min sec)	Recalcification time (sec)	Factor VIII (AHF) (% of normal)
1 9	4	16	–	198	600

B Plasma fibrinogen and fibrinolytic activity in patient E S during study

Day after injection	Fibrinogen (g/100 ml plasma)	Fibrinolytic activity (µg/ml/hr) pH 6 3
0	0 31	0
7	0 40	Total lysis
9	0 16	Total lysis
11	0 13	Total lysis
14	0 058	0

treated during the study with corticosteroids It has been shown (23) that such compounds increase the AHI level in blood and shorten the coagulation time (9), an effect also demonstrated in cirrhotic patients (10) In this connection it is interesting to note that Fletcher et al have found that patients with cirrhosis of the liver show an abnormal response to nicotinic acid fibrinolytic activity being more easily produced and the effect more prolonged than in normals (11) They suggested that this phenomenon was due to impaired clearance of plasminogen activator by the liver It might be that not only fibrinolytic activators but also coagulation active components are not cleared in liver parenchymal injury Thus, it was found by Spaet et al (28) that in dogs blockade of RES resulted in failure to clear thromboplastin and thrombin injected into mesenterial veins

The two patients with epidermolysis bullosa hereditaria were investigated because of previous findings of a shortening of the whole blood clotting time and of a resistance to intravenously administrated heparin in this disorder (3, 12) No shortening of the fibrinogen half life was, however, observed

Summary

The turnover rate of ^{131}I labelled fibrinogen in normal subjects and in patients with different diseases was studied The

Factor IX (% of normal)	Factor X (% of normal)	One stage prothrombin time (patient/normal)	Factor II + factor VII (% of normal)	Fibrinogen (g/100 ml plasma)	Fibrinolytic activity μg/ml/h pH 6.3 pH	Platelets /mm 10
6[illegible]	103	17.0/14.0	85	0.58	23	10

TABLE XI Fibrinogen and fibrinolytic activity in all patients

Diagnosis (case no)		Fibrinogen — g/100 ml plasma: Before I	Range during study		Fibrinolytic activity: Before I pH 6.3	pH [illegible]	Range during study pH	
Hemophilia A	(1)	0.27	0.24	0.[illegible]7	24	0	0	0
Hemophilia A	(2)		0.27	0.35			0	4
von Willebrand's dis	(3)	0.2[illegible]	0.23	0.28	4	14	0	2
Hypoproconvertinemia	(4)	0.44	0.31	0.50	100	0	[illegible]	9[illegible]
Hypofibrinogenemia	(5)	0.09	0.05	0.09	0	0	0	150
Posthep cirrhosis	(1)	0.33	0.26	0.34	0	0	0	8
Posthep cirrhosis	(2)	0.17	0.13	0.17	130	127	0	50
Posthep cirrhosis	(6)	0.26	0.29	0.45	120	0	0	633
Posthep cirrhosis	(7)	0.28	0.20	0.31	0		0	154
Alcoholic cirrhosis	(3)	0.[illegible]7	0.23	0.30	40	0	0	64
Alcoholic cirrhosis	(4)	0.55	0.40	0.55	0	294	0	400
Alcoholic cirrhosis	(8)	0.21	0.14	0.23	0		0	234
Fatty liver	(5)	0.47	0.38	0.58	0	0	0	17
Polycythemia vera	(1)	0.24	0.15	0.26			0	80
Polycythemia vera	(2)	0.28	0.20	0.24	70	37	0	4
Polycythemia vera	(3)	0.35	0.28	0.33	0	0	0	40
Polycythemia vera	(4)	0.31	0.26	0.34	34	0	0	77
Polycythemia vera	(5)	0.29	0.23	0.30	0	0	0	0
Polycythemia vera	(6)	0.42	0.33	0.37	0	183	0	57
Epidermolysis bullosa		0.27	0.21	0.28	114		17	2[illegible]8
Epidermolysis bullosa		0.30	0.21	0.32	0		0	[illegible]8
Normals		0.[illegible]6 ± 0.0[illegible]			0–100	0–100		

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Serum Lipids in Renal Artery Stenosis and Other Hypertensive States

I Abdominal Aorta, Renal Arteries and Fasting Serum Lipid Levels

By

B Hood, I Brolin, H Kjellbo and G Angervall

Renal artery stenosis offers the clinical investigator a unique opportunity to study the relationships between hypertension, atherosclerosis and other varieties of arterial disease The fact that surgery is commonly performed in this condition also permits the study of relationships between atherosclerotic stenosis in the main stem or the main branches of the renal artery and arteriolosclerosis in the peripheral renal vascular bed Surgery also gives the opportunity of confirming or rejecting the radiological impression of the etiology of the arterial disease The study of atherosclerosis in this particular region seems therefore more promising than in other parts of the arterial system

During the last three years all cases of renal artery stenosis were investigated with serum lipid measurements and the majority with fat tolerance tests as well For comparison similar investigations were simultaneously started in cases of other hypertensive states

Clinical and radiological examination Clinical material

All cases with lesions of the renal artery of doubtful hemodynamic significance have been excluded in order to facilitate the comparison between established renal artery stenosis and other varieties of hypertension The diagnostic examination of all these groups was extensive including besides intravenous pyelography and other routine checks for hypertension abdominal aortography and also repeated pitressin tannate tests quantitative examination of sediments and quantitation of bacteriuria In the majority of the cases micturition urethrocystography was also performed In the vast majority of cases in the three clinical groups blood pressure was measured in both arms and both legs Auscultation was done regularly over the cervical abdominal and usually also over the sacral regions

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Abdominal aortography was performed with contrast medium injection through a catheter introduced through the femoral artery by the Seldinger technique The exposures were made with stereo tubes with a frequency of 3—6 sec Selective arteriography was of limited help and was done as a complementary investigation in a few cases only

The radiograms were interpreted independently of the radiologist who at the time was not aware of the findings from the other areas of the investigation The appearance of the aortic wall was graded according to a simple system In this system grade 0 would mean a completely smooth abdominal aortic outline grade + would mean small irregularities of the aortic outline grade ++ rather extensive and wide spread changes of the outline and grade +++ a completely irregular outline covering the whole length of the abdominal aorta and leading to irregular widenings and constrictions of the aorta

Renal arterial lesions were divided according to whether they appeared in the proximal or the peripheral part in relation to bifurcation of the main renal artery The severity of the stenosis was graded into four stages where grade IV means complete occlusion grade III severe or sub-total stenosis grade II marked stenosis with post stenotic dilatation and grade I slight but definite constriction Lesions of severity grade I have been excluded in the results below in order to exclude border line lesions In the presentation of the results for the sake of simplicity the remaining grades II III and V have been lumped together in one group In the two groups of renal artery stenosis and that with hypoplasia of the kidney the examination also included elaborate split kidney function tests under varying experimental conditions Further confirmation of the diagnosis was achieved at operation in a large number of cases of renal artery stenosis and in some cases of hypoplasia of the kidney The series of established renal artery stenosis includes cases with unilateral and bilateral main stem stenosis, and with marked stenosis or occlusion of larger branches of the renal artery Aneurysms of the renal artery of uncertain etiology were excluded The control group of hypertensives is somewhat sparsely represented between 50 and 65 years of age Patients with essential hypertension in these age groups are nowadays only rarely admitted if they do not exhibit some serious complication, such as would exclude them from the studies necessary for the present work Moreover minor renal artery lesions are rather common in these age groups and such cases were excluded for reasons given above

The clinical material was subdivided as follows

A *The control group* consisted of 78 clinically healthy individuals, 42 males and 36 females representing all decades from 20 to 80 years The examination included ECG and detailed questioning about dietary habits Only individuals on ordinary diets were accepted These individuals were proven to be without signs of cardiac disease hypertension diabetes (although glucose tolerance tests were not done routinely) and chronic or acute infection at the time of the sampling Individuals with a history of cholelithiasis were excluded

B *The group of renal artery stenosis* was sub-divided as follows

1 Proximal renal artery stenosis 81 cases The lesions involved the ostium itself whether or not they extended for a distance into the renal artery These lesions are dominated by atherosclerosis according to our experience and that of other authors (referred to in the discussion From this group of 81 we have in table I subtracted a *Ia* mixed group with unusual radiological and clinical features 14 cases There were 10 patients with multiple involvement of either cervical subclavian coronary or iliac arteries as well as the renal arteries Among these patients there were three cases with Leriche syndrome in addition to the above features The group also included two cases with distal aortic aneurysm the only ones in all the groups) and two

TABLE I Serum cholesterol and serum glyceride glycerol in healthy controls in sub-groups of renal artery stenosis and in other hypertensive states

		No	Cholesterol	Differs from healthy controls	Glyceride glycerol	Differs from healthy controls
Healthy controls	♂	42	255±6		1.14±0.06	
	♀	36	279±8		1.00±0.06	
Renal artery stenosis					n 51	
Proximal	♂	50	259±7		1.55±0.09	Sign. higher
	♀	31	288±13		1.57±0.13	Sign. higher
Mixed group with unusual *radiological and clinical* features	♂	*7*	*215±11*	Sign. lower	*1.06±0.10*	
	♀	7	302±34		1.66±0.3[illegible]	
Proximal minus mixed group					n 45	
with unusual radiological and clinical features	♂	44	264±7		1.61±0.10	Sign. higher
	♀	24	285±14		1.55±0.14	Sign. higher
Peripheral	♂	20	273±9		1.35±0.08	Sign. higher
	♀	16	247±14		1.21±0.08	Sign. higher
Non-atherosclerotic group (fibromuscular+cone shaped stenosis)	♂	5	243±12		1.32±0.18	
	♀	13	211±17	Sign. lower	1.07±0.08	
Other hypertensive states	♂	34	234±8	Sign. lower	1.35±0.10	
	♀	27	254±9	Sign. lower	1.14±0.08	

One male case was transferred from the total proximal to the mixed group due to discovery of multiple artery lesions. This was done after completion of the statistics. It does not materially influence the mean value of the respective groups.

cases with histologically proven arteritis. As this group seems of considerable interest they will be described in more detail (5).

2. Peripheral renal artery stenosis: 44 cases. These lesions involved the middle or the third part of the main renal arteries. They might be multiple and they often involved the first branches of the renal arteries. This group contained one group of definitely non-atherosclerotic character outlined below and also cases suspected or proven to be of atherosclerotic origin.

The non-atherosclerotic group comprised 18 cases (group 2a). To this group cases with periarterial fibrosis (6) were referred; there were also 12 cases of fibromuscular hyperplasia included in this group. The mean age of this group was 37 years.

C. *Hypertensive disease — other causes*. This group of 63 cases included essential hypertension, hypoplasia of the kidney and chronic pyelonephritis with hypertension. All patients with serum creatinine above 2.5 mg % were excluded.

Chemical method

The individuals were on an ordinary diet when the blood studies were performed. Blood was drawn in the fasting state for cholesterol, triglyceride and turbidity measurements in serum.

TABLE II Frequency of elevated glyceride glycerol values i e >1 8 mMol/L, in healthy controls, renal artery stenosis and other hypertensive states

		Total no	Glyceride glycerol >1 8 mMol/I Number	
Healthy controls	♂	42	4	♂ + ♀ = 6/78 = 8%[1]
	♀	36	2	
Proximal stenosis	♂	50	11	♂ + ♀ = 19/81 = 23 5%[2]
	♀	31	8	
Peripheral stenosis	♂	20	2	♂ + ♀ = 2/36 = 6%[3]
	♀	16	0	
Other hypertensive states	♂	33	7	♂ + ♀ = 10/60 = 17%[4]
	♀	27	3	

1—2 significant 1—3 not significant 1—4 not significant 2—4 significant

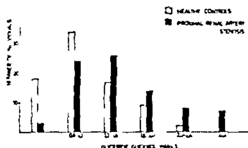


Fig 1 Distribution of serum glyceride glycerol in proximal renal artery stenosis compared with controls. Note the shift towards higher levels in proximal renal artery stenosis

Cholesterol was measured by the Cramér and Isaksson (3) modification of the Theorell procedure and triglyceride with the Carlson (1) modification of the method of Carlson and Wadström (2) Turbidity was measured in the Beckman B spectrophotometer as the extinction at 650 $\mu\mu$ with 1 cm light path

Results

The material as a whole and its major sub-groups were divided according to sex and age (above and below 50) When no significant differences appeared as regards serum lipid parameters measured sex and age have been left out of consideration in some of the tables and diagrams in the subsequent analysis

Table I gives the mean values of serum cholesterol and glyceride-glycerol for all the clinical groups None of the groups showed significantly higher mean cholesterol values than the control groups Males in the mixed group, females in the non atherosclerotic group and both sexes among the hypertensives had significantly lower average cholesterol values but the mean age in the female non atherosclerotic group was significantly lower than in the controls

For both males and females the glyceride glycerol levels however, were significantly higher in proximal stenosis and just significant at the 0 05 level in peripheral stenosis as compared with the controls The group of hypertensives as well as the non atherosclerotic group of renal artery stenosis had glyceride glycerol values somewhat higher than the controls but not significantly so

TABLE III Serum lipid parameters and radiological appearance of aorta in renal artery stenosis and other hypertensive states

Group		Aorta grade 0	Aorta grade +	Aorta grade ++ and +++	Total
Renal artery stenosis	n	9	23	49	81
A Proximal	Mean age	46±3 6[1]	45±1 4[2]	55±1 1	
	Cholesterol	282±16	259±10	273±10	270±7
	Glyceride glycerol	1 73±0 39	1 47±0 09	1 56±0 10	1 55±0 07[10]
B Peripheral	n	17	15	4	36
	Mean age	47±3 1[4]	46±3 7[5]	50±5 1	
	Cholesterol	259±13	256±13	294±14	262±8
	Glyceride glycerol	1 25±0 07	1.23±0 09	1 69±0 20	1 79±0 06[11]
Other hypertensive states	n	36	18	6	60
	Mean age	38±1 8[7]	48±1 9[8]	55±2 6	
	Cholesterol	244±9	234±10	266±24	243±6
	Glyceride glycerol	1 21±0 08	1 29±0 12	1 47±0 32	1 26±0 0[illegible] [12]

[1]—[2] not significant
[1]—[3] significant
[2]—[3] significant
[4]—[5]— not significant
[7]—[8] significant
[7]—[9] significant
[8]—[9] significant
[10]—[11] significant
[10]—[12] significant

When the group with unusual radiological and clinical features (multiple arterial stenosis distal aortic aneurysm, arteritis) was removed from the main group of proximal renal artery stenosis the mean glyceride glycerol values were somewhat higher in the remaining male group

The frequency of high values i e glyceride glycerol values above 1 8 mMol/L was 23 5 % in the group of proximal stenosis and this differed significantly ($p < 0.05$) from the group of control cases (8 %) and peripheral stenosis (6 %) In the group containing other varieties of hypertensive disease 17 % had values above 1 8 mMol/L This represents a significant difference from the controls (table II) The distribution of glyceride glycerol values in proximal renal artery stenosis and controls has been given in fig 1

When the material in the three main aortographed groups were divided according to the radiological grading of the aorta (table III and fig 2), those with marked and severe involvment (grade ++ and +++) of the aorta were of a significantly higher age both in the group of hypertensives and in that

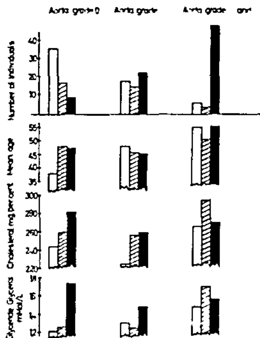


Fig 2 Striped columns peripheral renal artery stenosis black columns proximal renal artery stenosis and white columns other hypertensive states Caution broken columns and scales for mean age serum cholesterol and serum glyceride glycerol

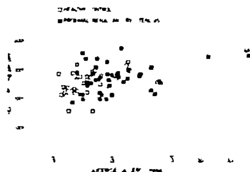


Fig 3 Black quadrates male patients with proximal renal artery stenosis (the group with unusual clinical and radiological features subtracted Open quadrates controls Only 7 patients to the left of the median glyceride glycerol of controls Of these 2 definitely hypercholesterolemic and 1 case with borderline cholesterol values

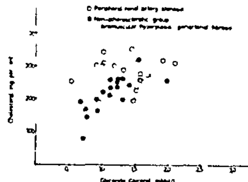


Fig 4 This figure contains 44 patients with peripheral renal artery stenosis In some of the tables the number of 36 has been given 8 cases of fibromuscular hyperplasia have been added after completion of the statistics The statistical calculations were not repeated due to the obviously heterogeneous character of the material

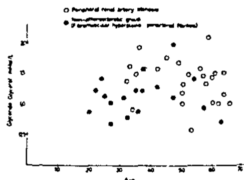


Fig 5 Note the concentration of cases with fibromuscular hyperplasia and periarterial fibrosis in younger age groups

of proximal renal artery stenosis Cholesterol did not differ significantly between any of the groups while the mean glyceride glycerol value from the whole group of proximal renal artery stenosis differed significantly from the hypertensive and peripheral renal artery stenosis groups The most striking finding was that while marked and severe involvement of the aorta was a frequent finding in proximal renal artery stenosis it was rather rare in the groups of hypertensives and of peripheral renal artery stenosis

In fig 3 the distribution of cholesterol and glyceride glycerol within the male

group of proximal renal artery stenosis is shown after subtraction of the cases with unusual clinical and radiological features As is seen from the text only four were below the median value of glyceride glycerol in the control group and below the median of serum cholesterol Two of these cases were strongly suspected of having arteritis due to their clinical appearance but histological confirmation was lacking In figs 4 and 5 the non atherosclerotic group has been plotted against the rest of the peripheral group The serum cholesterol and glyceride glycerol levels although lower than the rest of the peripheral group distribute in a fairly normal manner when the younger age of this group is taken into consideration There was no apparent correlation to the height of the blood pressure in any of the groups

Discussion

The radiological appearance of the aorta showed that the degree of aortosclerosis was worse in patients with proximal renal artery stenosis than in the other groups This seems natural enough as this type of lesion often appears at operation or autopsy as an extension of abdominal aortic sclerosis into the first part of the renal artery From the works by Wylie and Wellington 1960 (10) McCormack 1961 (7) Hunt and co workers 1962 (6) Maxwell and Prozan 1962 (8) Wellington 1963 (9) and Holley and co workers 1964 (4) it seems apparent that the atherosclerotic lesions dominate heavily at the aortal orifice and in the first cm of the renal artery while the other more common varieties i e fibromuscular hyperplasia and periarterial fibrosis usually are situated in the middle part of the main renal arteries and further distally

The mean glyceride glycerol level was significantly higher in the whole group of proximal artery st nosi than in the two other main group and the controls The few patients wit n the groups of peripheral artery steno and hyperten sives of other varieties p esented ad vanced abdominal aorto e osi 4 and 6 individuals respectively ad somewhat higher glyceride glycerol el On the other hand age was al h gher a d significantly so in the h pe n es The limited number of hypert n e n the higher age groups -o years) is a serious drawback study As pointed out earlier one of the main reasons for t i a hat 26 patients 19 of which above th ag of 50 with minor renal artery le o of doubtful significance had to be excluded due to difficulties of interpretation These radiological findings on the other hand illustrate the fact that in elderly age groups as pointed out among others by Holley et al for autopsy material minor renal artery lesions are extremely frequent especially in hypertensive disease but also in normotensive individuals

In the 18 cases of definitely non atherosclerotic character taken out of the group of peripheral artery stenosis the females had a normal glyceride glycerol value and a serum cholesterol value significantly lower than in the female control group However the mean age in this group was low (37 years) and the figure accords well with

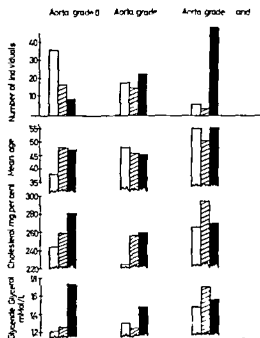


Fig 2 Striped columns peripheral renal artery stenosis black columns proximal renal artery stenosis and white columns other hypertensive states Caution broken columns and scales for mean age serum cholesterol and serum glyceride glycerol

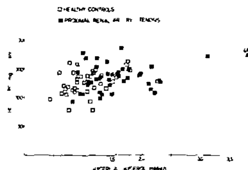


Fig 3 Black quadrates male patients with proximal renal artery stenosis (the group with unusual clinical and radiological features subtracted) Open quadrates controls Only 7 patients to the left of the median glyceride glycerol of controls Of these 2 definitely hypercholesterolemic and 1 case with borderline cholesterol values

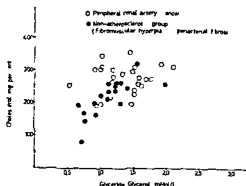


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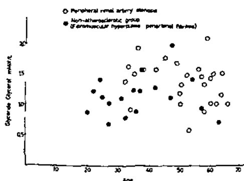


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In fig 3 the distribution of cholesterol and glyceride glycerol within the male

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Serum Lipids in Renal Artery Stenosis and Other Hypertensive States

II Renal Artery Stenosis with Unusual Radiological and Clinical Findings

By

B Hood I Brolin, H Kjellbo and G Angervall

In a previous study (5) we have discussed the serum lipid parameters in 125 cases of renal artery stenosis as compared with other hypertensive states and a control group Especially in proximal stenosis we found the average serum level of triglyceride estimated as glyceride glycerol to be significantly higher than in controls Within the 81 cases of proximal stenosis there was a group of 14 cases with distinct clinical and/or radiological features This group will be more closely discussed in the present paper

Methods

Clinical radiological and biochemical methods have been discussed in the preceding paper (5) The routine clinical examination of patients with hypertension of all varieties included auscultation over the neck and abdomen as well as blood pressure measurements on all four extremities

The group includes 10 cases of multiple artery stenosis i e lesions of at least two other arteries besides the renal artery such as the carotid subclavian vertebral axillary coronary or iliac arteries as well as the Leriche syndrome

Two cases of arteritis with giant and plasma cell infiltration are included in this group In one of the operated male case of multiple artery stenosis this picture was also present in the renal arteries The two cases of distal aortic aneurysm in connection with renal artery stenosis which we have encountered have also been referred to this mixed group of unusual cases

Results

It is seen from table I that of the three females who showed definite elevation of one or both serum lipid parameters two were rather elderly 57 and 59 years respectively The female of 46 had notably high cholesterol and glyceride glycerol values

The male EL 35 had both radiological evidence of multiple arterial stenosis and histological evidence of

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TABLE I Renal artery stenosis with complex radiological and clinical findings (Multiple occlusions aneurysm histologic findings of arteritis

	Pat	Age	
Females Multiple *arterial stenosis*	EK	57	Bilateral proximal renal artery stenosis Right axillary stenosis with collaterals Severe distal aortic atherosclerosis Conservative regimen *strict diet Hypotensive agents*
	KK	59	Xanthomatosis Bilateral renal artery stenosis Extreme abdominal aortosclerosis Multiple advanced lesions right common carotid right subclavian and right vertebral arteries Patho anatomical diagnosis wide spread severe atherosclerotic changes Coronary sclerosis Myocardial infarction
	ES	46	Left ventricular failure Angina pectoris Incomplete Leriche syndrome left subclavian stenosis Left nephrectomy 1956 Blood pressure 1956—1964 130/90—180/105 Normalisation of lipid parameters on strict diet and Atromid S
	EB	68	Complete occlusion right subclavian moderate stenosis left subclavian left vertebral left internal carotid and left renal arteries Sedimentation rate 69—110 mm Serum lipids show marked variation Polycythemia trombocythemia leucocytosis α^2 globulin 0 9 g % Temporal biopsy without remarks
	FJ	50	Complete occlusion left common carotid artery Moderate stenosis of the brachiocephalic trunk Proximal bilateral renal artery stenosis Sedimentation rate 60—91 AST 200 units AI P 4 units α^2 0 6 γ glob 1 6 g/100 ml Dead of uremia Multiple cerebral infarctions
	EL	49	Complete Leriche syndrome Complete occlusion left brachial artery Moderately severe bilateral proximal renal artery stenosis
	KE	57	Total occlusion left subclavian left renal and inferior mesenteric arteries Narrow left vertebral artery Sedimentation rate 43—61 mm
Males Multiple arterial stenosis	KK	39	Left arm 150/125 Stenosis both subclavian and vertebral arteries right coronary artery both renal arteries proximal stenosis Complete Leriche syndrome Reconstructive operations left subclavian left vertebral and both renal aorta and iliac arteries Normotension on small doses of chlortalidon
	AW	59	Stenosis left internal carotid both vertebral and two of five renal arteries Total occlusion right carotid artery Sedimentation rate 12—29 mm
Multiple arterial stenosis + arteritis	EL	35	Right subclavian stenosis left iliac artery stenosis bilateral proximal *renal artery stenosis Malignant hypertension Bilateral reconstructive* renal artery operation Microscopy degenerative medial changes with focal necrotic changes Infiltration of lymphocytes and giant cells
Distal aortic aneurysm	SW	48	*Distal aortic aneurysm Multiple stenosis right main and left upper renal* arteries Reconstructive operation 1961 Consistently normotensive after operation

of cervical arteries Leriche syndrome distal aortic

Serum lipids					
Chol	Glyceride glycerol	Extinct at 650 mμ	Furth	Blood pressure range	Aorta renal grade
352	1 50	0 10	II	190/100 110/90	++
355	2 96	0 09	II	320/140 265/120	+++
430	2 62	0 14	II	230/130 250/140	++
270	1 75	0 12	II	270/140 155/100	++
212	0 74	0 07	IV	240/95 270/115	+++
273	1 22	0 10	II	250/130 230/110	+++
199	0 81	0 07	II	142/39 Intraaortal press	++
198	1 01	0 09	II	200/110 220/110	++
221	1 36	0 08	II	230/160 200/100	+++
162	1 07	0 07	IV	220/160 240/180	++
204	0 66	0 07	II	250/130	+++

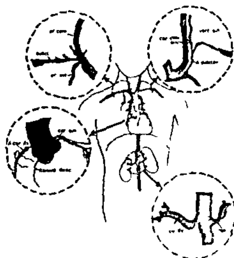


Fig 1 Schematic representa... arterial lesions in KK male 39 ... case in table I)

arteritis while in the mal... classified as arteritis the arteritic ... ment was such that he might well ... shown a similar radiological picture ... he been investigated while alive

Fig 1 shows a semi schematic representation of all the stenoses encountered in the extensive radiological examination of the male case KK 39 The study included aortic arch angiography front view abdominal aortography and coronary angiogram No side view projections of the abdominal aorta were performed We have estimated at least ten vessels to be stenotic the majority of these at the branching off points

In fig 2 the serum lipid values of this mixed group of cases have been plotted together with the cases of proximal renal artery stenosis without such distinctive features The majority of the cases belonging to the mixed group are below the median values for the whole

Table I Cont

	Pat	Age	
	TL	43	Right proximal renal artery stenosis Distal aortic aneurysm Resection of aneurysm right nephrectomy Post-op blood pressure 170/105—215/125 Chlorothiazide 25 mg × 2 guanethidine 10 mg × 3
Arteritis	TS	40	Twice hospital admitted 1949 and 1950 for suspected kidney rupture (football game) Left proximal renal artery stenosis Reconstructive operation Inflammatory changes with lymphocytes plasma cells and giant cells Consistently normotensive after operation
	BS	43	Angina pectoris since the age of 35 Bilateral renal artery stenosis Sudden cardiac death Thoracal and abdominal aorta both carotid subclavian and renal arteries show severe atheromatous lesions and general arteritic changes Mononuclear lymphocytic and plasma cell infiltration Considerable adventitial fibrosis Severe adrenal tuberculosis with necrosis Multiple myocardial infarctions

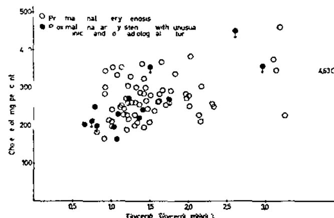


Fig 2 Serum cholesterol and serum glyceride glycerol in 14 cases of proximal renal artery stenosis with unusual clinical and radiological features (black circles) plotted against the background of proximal renal artery stenosis without such distinctive features (open circles)

Note Majority of cases of the black symbols concentrate in the lower region as regards both cholesterol and glyceride glycerol

group of proximal renal artery stenosis Two of the open circles in the left lower corner of the diagram may very well represent cases of arteritis

Clinically they had myalgia, marked elevation of sedimentation rate and high fibrinogen levels However, in the absence of radiological evidence of multiple arterial involvement or histologic evidence of arteritis, they should remain in the main group of proximal renal artery stenosis

Serum lipids					
Chol	Glyceride glycerol	Extinct at 650 mμ	Fundi	Blood pressure range	Aorta radiol grade
241	1 41	0 06	III	255/155 210/135	+++
250	0 79	0 06	IV	220/140 195/125	0
228	1 09	0 08	II	240/140 180/140	++

Discussion

This group of cases with unusual features in addition to proven renal artery stenosis is too small and too mixed to allow definite conclusions However, it seems suggestive that the severe picture of widespread vascular disease may be obtained in cases either with elevated (the minority) or with definitely low lipid levels The possibility that active arteritis may lower the lipid levels must be considered In our experience multiple arterial involvement of the degree seen in this series seems rare in hyperlipidemic states We have just completed a follow up study of 460 patients originally selected because of serum cholesterol levels of 300 mg per cent and above with observation times between 5 and 18 years 40 % of these had borderline or definitely high serum glyceride glycerol levels In 260 of the survivors a thorough clinical examination, including blood pressure measurements in both arms, failed to show any signs of clinically important disease of arm or neck vessels (11)

Two different lines of pathogenesis for unusually widespread vascular disease in middle age or early middle age might be suggested The question should be pursued more systematically on the following lines — Liberal indications for aortic arch angiography and coronary angiograms should be applied in proven renal artery stenosis, and for renal angiography in cases with proven aortic arch syndromes Systematic studies of serum lipid levels serum electrophoretic examination and search for rheumatoid factors should be done More liberal use should be made of the temporal artery biopsy More systematic study should be made of the medial and adventitial layers of renal artery biopsies in the patients operated upon even in cases where there are definite atheromatous deposits of the intima

Summary

1 Out of a group of 125 patients with renal artery stenosis in whom serum lipid studies were done, 81 had proximal lesions From this latter group 14 cases were selected because of their distinctive clinical and/or radiological features, predominantly multiple arterial involvement

2 Three females of this group had elevated serum lipid levels The rest — particularly the younger males — presented levels in the low normal range

This is in contrast to the significantly high glyceride glycerol value found in the whole group of proximal renal artery stenosis as reported earlier (5)

3 The relations between multiple arterial involvement and widespread arteritis have been touched upon Far more systematic studies must be performed on a large material to settle the question whether there are at least two distinct lines of pathogenesis for severe widespread artery disease in early middle age

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Serum Lipids in Renal Artery Stenosis and Other Hypertensive States

III The Results of Fat-tolerance Tests

By

B Hood I Brolin H Kjellbo and G Angervall

In a series of hypertensives of not too closely specified origin Berkowitz (2) found a decreased fat tolerance using triolein both peroral and intravenous 131 I In a previous paper (4), we described a significantly raised mean value for serum triglyceride in patients with proximal renal artery stenosis as well as a significantly greater number of individuals exhibiting elevated values than in controls A group of patients with peripheral renal artery stenosis and hypertensive disease of other varieties had average triglyceride levels which showed a rise of borderline significance at the 0 05 level In the two latter groups the number of individuals with elevated values was not significantly different from the control

The finding reported by Berkowitz of a high frequency of pathological fat tolerance tests in hypertension also was restudied in part of our series in order to elucidate whether different groups of hypertensives exhibited a different response

The response to a fat load has been shown to be correlated to the fasting triglyceride level (1, 3) in most clinical groups However, Angervall in 1964 found for a small group (13) of normoglyceridemic, normo cholesterolemic males with clinical evidence of atherosclerosis, an exaggerated rise in the four hour value after an ordinary fat load as compared with all the other clinical groups investigated This group contained a number of individuals with renal artery stenosis Hood (1964) in a group of 41 such male subjects found in all three groups with fasting glyceride glycerol levels of < 1 0 1 0—1 5 and 1 5—2 0 mMol/L respectively higher responses than in controls with the same fasting levels (unpublished)

Clinical material and clinical methods have been discussed in a previous publication (4)

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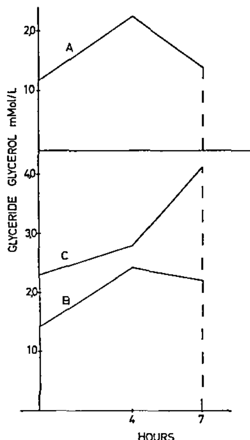


Fig 1 Curve A average response to a fat load in the group of controls males Curves B and C different types of response in normoglyceridemic (B) and hyperglyceridemic (C) male individuals with proximal renal artery stenosis Broken lines to the right serve to illustrate the areas which have been used for calculation of the surface areas in table I

The fat tolerance test was performed in 174 subjects as divided below

Controls	44
Proximal renal artery stenosis	57
Peripheral renal artery stenosis	26
Hypertensive disease of other varieties	47

After an overnight fast the fat load was given as 200 ml of heavy cream (80 g fat) Venous blood was sampled at 0, 4 and 7 hours centrifuged and analyzed for cholesterol and glyceride glycerol as in the previous paper (4)

Results

The assessment of the results of fat-tolerance tests have earlier been made in different ways Angervall (1) used the 4 and 7 hour values for glyceride glycerol, serum turbidity, cholesterol and phospholipid after a standardized fat meal as separate points for analysis in studying the behavior of different groups of subjects This type of study will register a fat tolerance test as not significantly different from controls if the values at the two points chosen just fail to differ significantly from the normal range

In the present study however, we have chosen to use both points for a simultaneous analysis by calculating the area covered by the values of the fat tolerance test according to fig 1

Table I shows that patients of both sexes in the proximal renal artery stenosis group had a significantly abnormal pattern in the fat tolerance test For peripheral renal artery stenosis and for hypertensive diseases of other varieties, the two sexes combined barely reached significantly pathological values while separately they failed to do so In an attempt to analyze whether the impaired fat tolerances for the various clinical groups were merely a function of the raised fasting glyceride glycerol levels, or whether they were due to exaggerated responses in individual patients individual data were plotted together with the range representing, for the 44 controls, the regression line $\pm$ 2 standard deviation for 7 hour glyceride glycerol upon the fasting glyceride glycerol level (fig 2) The figures for the 4th hour samples gave a similar picture but the

TABLE I Fat tolerance test in renal artery stenosis and other hypertensive states Results given in relative surface areas as calculated from the glyceride-glycerol values at 0 4 and 7 hours

Patient groups		No	Surface area	Differs from controls
Controls	♂	24	11 82 ± 1 00	
	♀	20	10 14 ± 0 90	
	♂ + ♀	44	11 06 ± 0 69	
Proximal stenosis	♂	36	15 62 ± 1 21	Sign
	♀	21	13 34 ± 1 04	Sign
	♂ + ♀	57	14 78 ± 0 86	Sign
Peripheral stenosis	♂	15	13 81 ± 0 93	
	♀	11	12 51 ± 1 34	
	♂ + ♀	26	13 26 ± 0 77	Sign
Other hypertensive states	♂	29	14 57 ± 1 28	
	♀	18	11 96 ± 1 15	
	♂ + ♀	47	13 57 ± 0 94	Sign

7th hour figures are considered to be more representative of the removal phase of the fat tolerance As is clearly shown 15 % (20/130) of all the hypertensive subjects tested showed an exaggerated response The number of patients falling within ± 2 standard deviations and above or below is seen in the legend to fig 2 The exaggerated response was less evident in the subjects with peripheral stenosis than in the two other groups However, the group of peripheral renal artery stenosis contained more females than males (4) The slopes of the regression lines in the two sexes for the glyceride glycerol at 7 hours upon the zero values (fasting) showed a significant difference, the slope being definitely steeper in the males (fig 3)

Discussion

Berkowitz observation of an impaired fat tolerance in hypertensives has been confirmed with a method differing from his The impairment has been shown to appear in all kinds of hypertension investigated, i e proximal renal artery stenosis, peripheral renal artery stenosis and other types of hypertension Analyzed according to the area under the fat removal curve the impaired fat tolerance seemed more pronounced in proximal renal artery stenosis — as might be expected from the higher average glyceride-glycerol level in this group However, all three clinical groups contained some definitely normo glyceridemic subjects who showed an exaggerated response to the fat load The response to the fat load was in males with proximal

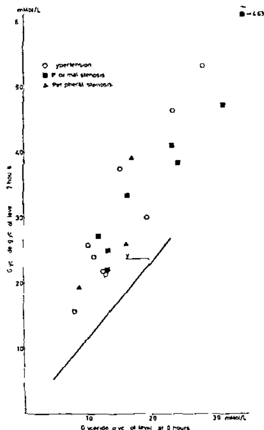


Fig 2 Individua s showing exaggerated response to fat loads plotted against the background regression line ± 2 δ based on 44 controls Distribution of observations (above + 2 δ within ± 2 δ below — 2 δ) proximal stenosis 8 4 › 4 peripheral stenosis 3 22 1 hypertension of other varieties 9 36 2

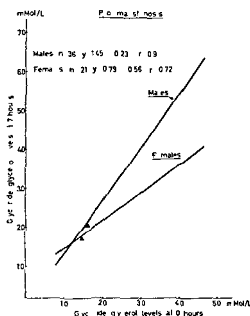


Fig 3 Regression lines for 7 hour values of glyceride glycerol upon zero hour values (fasting) after 80 g fat The slope for the males is signif icantly steeper than that for the females

stenosis significantly more abnormal than in the females at the same fasting glyc eride glycerol values In the other two clinical groups no significant difference between the two sexes appeared in this respect

The mechanism behind this impaired fat tolerance in hypertensives of different cathegories remains unknown In hyper tensives, particularly in proximal renal artery stenosis there were higher fasting glyceride glycerol levels and higher, more prolonged and sometimes exag gerated elevations after a fat meal as compared with controls What role these features play in combination with the elevated pressure for the acceler ated atherogenesis is difficult to evaluate, but they might be of decisive importance for the development of vascular damage

Summary

1 The response to a fat load is signif icantly abnormal in all types of hyper tension investigated in proximal and peripheral renal artery stenosis and in other types of hypertension This seems more marked in proximal renal artery stenosis, which is in accordance with

the higher fasting glyceride glycerol levels found in this group

2 In all three clinical groups there were individual exaggerated responses to the fat load even in subjects with normal or low normal fasting glyceride-glycerol

3 The impaired fat tolerance pattern in proximal renal artery stenosis was significantly more pronounced in males than in females

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Transmission of Murmurs within the Heart

A Contribution to the Interpretation of Intracardiac Phonocardiography

By

ALF WENNEVOLD

It has been widely accepted that at intracardiac phonocardiography the murmurs are recorded only in the ostium or defect in which they originate and along the blood stream away from their point of origin Furthermore, murmurs originating in the right side of the heart and circulation are not recorded in the left side, and vice versa The interpretation and diagnostic conclusions from the results of intracardiac phonocardiography have been based on these assumptions

However, exceptions do exist (1—8) and the purpose of this paper is to demonstrate that regurgitant murmurs of aortic insufficiency may be transmitted to the right ventricle and that ejection murmurs from the aorta and from the right pulmonary artery may be transmitted to the superior caval vein and the right atrium It will be shown that the diagnostic value of intracardiac phonocardiography is increased through recognition of the fact that murmurs of the left side of the heart may be transmitted to the right side of the heart

Submitted for publication October 19 1965

Methods and materials

The Allard Laurens micromanometer has been used to record pressures and intracardiac phonocardiogram (10, 11) From November 1963 to 1 1 1965 right heart catheterization with intracardiac phonocardiography has been performed in a total of 138 patients mostly young adults and children over 7 years old In this study are included all 17 cases in which a systolic murmur was recorded in the superior vena cava Two of these patients with predominant aortic insufficiency and with both a systolic and a diastolic aortic murmur also had a diastolic murmur recorded in the right side of the heart

Results

Transmission of regurgitant murmurs of aortic insufficiency to the right side of the heart

In 2 out of 6 patients with aortic insufficiency the diastolic murmur was recorded in the inflow tract of the right ventricle (table I), in one of them it was also recorded in the superior caval vein

TABLE I Diastolic and systolic murmurs recorded in the right side of the heart in patients with predominant aortic insufficiency

Case no	Diagnosis	Intensity of diastolic intracardiac murmur in: Right ventricle (mm Hg)	Superior caval vein (mm Hg)	Intensity of systolic intracardiac murmur in: Superior caval vein (mm Hg)	Right atrium (mm Hg)	Right pulm artery (mm Hg)	Main pulm artery (mm Hg)
7 369	AI + bicuspid valve	0 4	—	0 4	0 4	0 4	—
7 207	AI + MI	0 1	0 2	0 1	0 1	0 2	0 2

AI = aortic insufficiency MI = mitral insufficiency

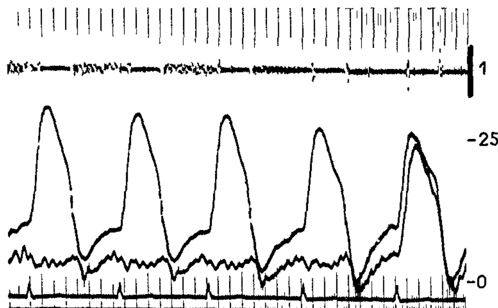


Fig 1 Intracardiac phonocardiogram showing diastolic murmur in the inflow tract of the right ventricle in a man with aortic insufficiency (case no 7369) In the upper right corner the calibration signal corresponding to pressure variations of 1 mm Hg is marked with a black vertical line The upper pressure tracing records the pressure through the micromanometer at the tip of the catheter synchronously with the sound the lower pressure tracing is recorded through the side hole 1 5 cm from the tip and is calibrated to 0 and 25 mm Hg Left the side hole is seen to be situated in the right atrium right the side hole now is in the right ventricle while the diastolic murmur is diminished after the slight movement of the catheter

Case no 7369 Male 22 years old On auscultation a harsh systolic ejection murmur was heard over the base of the heart with maximum grade 3,6 in the 2nd and 1st right intercostal spaces In the 3rd left and 2nd right intercostal spaces a very faint high frequency early diastolic decrescendo murmur was heard A combined right heart catheterization and transseptal left heart catheterization was performed from the saphenous vein Normal pressures were found throughout the heart and in the aorta and no gradient could be demonstrated over the aortic valve Shunts were excluded through determination of oxygen saturation and use of the hydrogen electrode In the right pulmonary artery and in the superior caval vein a systolic ejection murmur was registered (table I) In the inflow tract of the right ventricle with the tip of the catheter close to the ventricular septum but lying freely in the cavity a faint systolic and a somewhat louder diastolic murmur was registered (fig 1) No diastolic murmur was recorded just below the pulmonary valve Aortography demonstrated bicuspid aortic valve with slight aortic insufficiency

Case no 7207 Male 11 years old with severe rheumatic aortic insufficiency and also slight mitral insufficiency At right heart catheterization the pressures were within normal limits and no shunts were found In the right and in the main pulmonary arteries a faint systolic ejection murmur was registered (table I) in the inflow tract of the right ventricle faint diastolic vibrations were recorded In the superior caval vein and in the upper part of the right atrium both a systolic and a diastolic murmur were demonstrated (fig 2)

Transmission of ejection murmurs of aortic stenosis to the superior caval vein and right atrium

Right heart catheterization with intracardiac phonocardiography was performed in 7 patients with aortic stenosis and in all a systolic murmur was recorded in the superior caval vein as well as at other sites (table II) A typical tracing is shown in fig 3 (case no 7299 In the 4 patients with valvular aortic stenosis the murmur was louder in the superior caval vein than in the right atrium while the murmur tended to be louder in the right atrium in the 3 patients with subvalvular or predominantly subvalvular stenosis As shown in table I a systolic aortic murmur was also recorded in the right side of the heart in the 2 patients with predominant aortic insufficiency

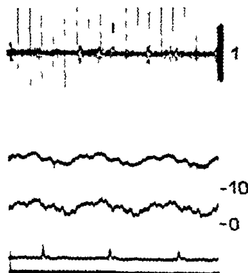


Fig 2 Intracardiac phonocardiogram showing systolic and faint diastolic murmur in the superior caval vein in a boy with severe aortic insufficiency (case no 7207)

Transmission of ejection murmurs from the right pulmonary artery to the superior caval vein

In 8 patients without aortic heart disease a systolic murmur was recorded in the superior caval vein and in 4 of the

TABLE II Systolic murmurs recorded in superior caval vein in patients with aortic stenosis

Case no	Diagnosis	Gradient across aortic valvular and/or subvalvular area (mm Hg)	Intensity of systolic intracardiac murmur in			
			Superior caval vein (mm Hg)	Right atrium (mm Hg)	Right pulm artery (mm Hg)	Main pulm artery (mm Hg)
7 101	Valvular AS+ AI+MS	25	0 8	0 3	0 2	0 3
7 043	Valvular AS+AI	Not measured	1 0	0 3	0 4	0 2
7 289	Valvular AS	35	0 8	0 5	0 3	0 3
7 299	Valvular AS	75	0 5	0 2	0 4	0 3
7,309	Subvalvular AS	35	0 1	0 7	0 5	0 2
5 222	Subvalvular+ valvular AS	21	0 1	0 2	0 4	0 3
6 676	Subvalvular AS	9	0 2	0 2	0 5	0 3

In none of the patients was any gradient found acros the pulmonary valve
AS—aortic stenosis MS—mitral stenosis

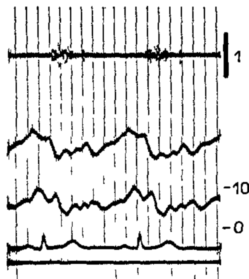


Fig 3 Recording of a systolic murmur in the superior caval vein in a man with valvular aortic stenosis (case no 7299)

patients also in the right atrium (table III) In 7 of the patients a rather loud murmur was recorded in the right pulmonary artery

In one of the patients (case no 7334) the diastolic component of the continuous murmur of a patent ductus was registered both in the right pulmonary artery and in the superior caval vein (figs 5 and 6)

Discussion

The diastolic murmur of aortic insufficiency transmitted to the right side of the heart was in both cases localized to a very restricted area in the right

TABLE III Systolic murmurs recorded in the superior caval vein in patients without aortic heart disease

Case no	Diagnosis	Intensity of systolic intracardiac murmur in: Superior caval vein (mm Hg)	Right atrium (mm Hg)	Right pulm artery (mm Hg)	Main pulm artery (mm Hg)
6 957	Valvular PS	0 1	—	0 8	6 0
4 635	Valvular PS+coarct. right pulm art.	0.3	0 2	1 0	3 0
7 146	Valvular PS+ASD	0 1	—	Not entered	4 0
7 393	Supravalvular PS+ASD	0 2	—	1 8	4 0
7.226	Coarct right pulm art.	0 3	0 2	1 2	0 2
7 396	ASD	0 3	0 2	0 8	1 0
7 350	Coarct. right pulm art +ASD	0 1	—	1 8	0 4
7 334	PDA	0 4	0 3	1 0	9 0

PS=pulmonary stenosis Coarct. right pulm art =coarctation of the right pulmonary artery
ASD atrial septal defect PDA=patent ductus arteriosus

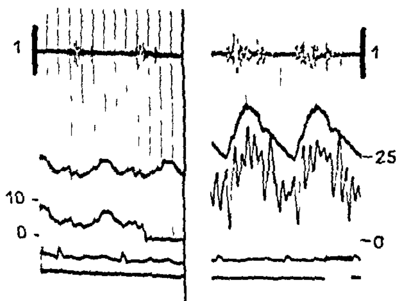


Fig 4 Intracardiac phonocardiogram in a girl with atrial septal defect case no 7396 Left systolic murmur in the superior caval vein. Right systolic murmur in the right pulmonary artery

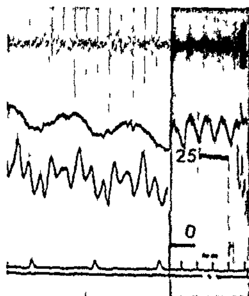


Fig 5 Continuous murmur recorded in the right pulmonary artery in a girl with patent ductus arteriosus (case no 7334)

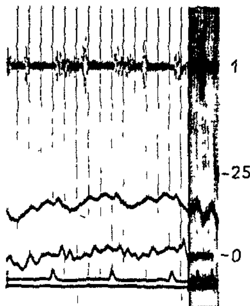


Fig 6 Continuous murmur in the superior caval vein in the same patient as in fig 5

ventricle and could easily have been missed This could explain why such a transmission has not been noticed previously

The transmission of the aortic systolic murmur to the right side of the heart has been reported by others (3, 4, 6, 8, 9) Feruglio (3) states that the murmur may be recorded in the superior caval vein in *severe* aortic valvular stenosis, while Reploh and associates (6) have found the transmitted murmur even in slight aortic stenosis The results in this paper indicate that even a systolic murmur due to aortic valvular disease without any demonstrable pressure gradient may be recorded in the superior caval vein (cf case no 7369) if the murmur has enough energy to be heard at external auscultation It seems as if the presence of aortic heart disease thus can be detected by sound recording during a simple right heart catheterization The relative intensity of the transmitted murmur recorded at the different sites in the right side of the heart (table II) may be used grossly to determine whether the aortic stenosis is valvular or mainly subvalvular, as was also reported by Reploh and associates (6)

As to the patients without aortic heart disease (table III), it seems justified to state that the rather loud murmur from the right pulmonary artery is transmitted to the superior caval vein due to anatomical contiguity of the vessels This assumption is substantiated by the recording of the continuous murmur of a patent ductus (case no 7334) all the way from the main trunk to the right branch of the pulmonary artery and in the superior caval vein

Differentiation between murmurs transmitted from the aortic area the subaortic area or the right pulmonary artery Though this study comprises but few patients, there seem to be rather consistent findings regarding the relative intensity of the murmurs (tables II and III) Accordingly, the intensity may be used in the differential diagnosis when a systolic murmur is recorded in the superior caval vein

In all the cases *without* aortic heart disease (table III) the murmur was loud in the right pulmonary artery (minimum 0 8 mm Hg) compared with the murmur in the superior caval vein (no more than 0 4 mm Hg), and was not always recorded in the right atrium

In the patients with *aortic valvular stenosis* (table II) the murmur was louder in the superior caval vein (minimum 0 5 mm Hg) than in the right pulmonary artery (no more than 0 4 mm Hg) In the patients with *aortic subvalvular* stenosis the murmur was faint in the superior caval vein and moderately loud in the right pulmonary artery, although the intensity here was definitely lower (no more than 0 5 mm Hg) than in those cases where the murmur was transmitted from the right pulmonary artery

Summary

In 17 out of 138 patients studied with intracardiac phonocardiography during right heart catheterization a systolic murmur was recorded in the superior caval vein

Furthermore in 2 of these patients the transmitted murmur of aortic insufficiency was registered in the inflow tract of the right ventricle

In 9 of the patients the systolic murmur in the superior caval vein was transmitted from the aortic or subaortic area whereas the murmur in the remaining 8 patients was transmitted from the right pulmonary artery

The transmission of systolic murmurs of aortic stenosis to the right side of the heart increases the diagnostic value of intracardiac phonocardiography as the presence or absence of aortic stenosis may be determined by a simple right heart catheterization The relative intensity of the murmur in the different sites of the right heart may help to differentiate between a murmur transmitted from the aortic valve from the subaortic area or from the right pulmonary artery

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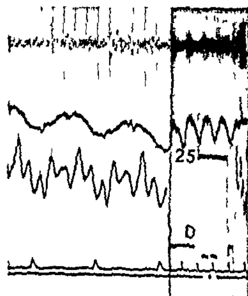


Fig 5 Continuous murmur recorded in the right pulmonary artery in a girl with patent ductus arteriosus (case no 7334

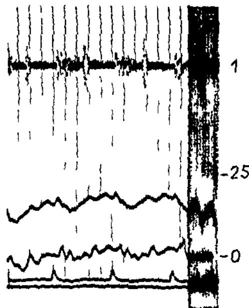


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ventricle and could easily have been missed This could explain why such a transmission has not been noticed previously

The transmission of the aortic systolic murmur to the right side of the heart has been reported by others (3, 4, 6, 8, 9) Feruglio (3) states that the murmur may be recorded in the superior caval vein in *severe* aortic valvular stenosis while Reploh and associates (6) have found the transmitted murmur even in slight aortic stenosis The results in this paper indicate that even a systolic murmur due to aortic valvular disease without any demonstrable pressure gradient may be recorded in the superior caval vein (cf case no 7369), if the murmur has enough energy to be heard at external auscultation It seems as if the presence of aortic heart disease thus can be detected by sound recording during a simple right heart catheterization The relative intensity of the transmitted murmur recorded at the different sites in the right side of the heart (table II) may be used grossly to determine whether the aortic stenosis is valvular or mainly subvalvular, as was also reported by Reploh and associates (6)

As to the patients without aortic heart disease (table III), it seems justified to state that the rather loud murmur from the right pulmonary artery is transmitted to the superior caval vein due to anatomical contiguity of the vessels This assumption is substantiated by the recording of the continuous murmur of a patent ductus (case no 7334) all the way from the main trunk to the right branch of the pulmonary artery and in the superior caval vein

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Free Fatty Acids of Plasma during Head-up Tilting (Orthostatic Stimulation)[1]

Studies in Normal Persons, Patients with Postural Hypotension and Anaesthetized Dogs

By

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The role of the sympathetic nervous system in the control of the cardiovascular system is well established (15 31) The sympathetic nervous system is also of importance for the mobilization of fat, in the form of free fatty acids (FFA), from adipose tissue into blood plasma 4 18, 20)

Recent studies have indicated that the sympathetic nervous system is differentiated with respect to cardiovascular control and to control of lipid mobilization (14 16 26, 27) During carotid occlusion and central vagal stimulation in anaesthetized dogs the blood pressure increases markedly with little or no rise in the arterial FFA level (14) The findings indicate that in these preparations, sympathetic vasoconstrictor nerves can be stimulated via baroreceptor reflexes without change in lipid mobilization

In the present investigation it was sought to study more closely the role of the baroreceptor reflexes in the mobilization of FFA Normal persons and anaesthetized dogs were tilted in the head up position to induce an orthostatic stimulation of the baroreceptor reflexes and the arterial levels of FFA and glycerol were followed The effect of orthostatic stimulation on plasma FFA was also studied in patients with disturbed sympathetic cardiovascular control (postural hypotension)

Material

Normal subjects (without postural hypotension)
Five men between 48 and 64 years of age (subjects 3 4 5 7 and 8 in table I) and six men between 19 and 21 years of age were studied They were normal and healthy as judged from routine clinical and laboratory investigations Exercise tests did not show any abnormalities with respect to maximal working capacity (28) or ECG

[1] A preliminary report was given in Lancet 2 594 1964

Submitted for publication November 11 1965

TABLE I Effect of head up tilting on the concentrations of FFA (mmoles/l) and glycerol (mmoles/l)

				Before tilting		
Subject no	Tilting angle	Tilting time		20 minutes	10	0
1	40	30	FFA	0 50	0 44	0 38
			Blood pressure	135	—	145
			Heart rate	68	68	60
2	40	30	FFA	0 52	0 47	0 51
			Blood pressure	125	125	130
			Heart rate	64	—	64
3	40	30	FFA	0 62	0 54	0 58
			Blood pressure	165	145	145
			Heart rate	72	70	68
4	40	30	FFA	0 50	0 54	0 45
			Blood pressure	165	—	160
			Heart rate	64	68	64
5	45°	20	FFA	0 48	0 50	0 52
			Blood pressure	130	130	130
			Heart rate	74	74	72
6	50	25	FFA	—	0 81	0 89
			Glycerol	—	0 080	0 091
			Blood pressure	—	110	110
			Heart rate	—	60	61
7	60	25	FFA	0 52	0 51	0 43
			Glycerol	0 089	0 079	0 068
			Blood pressure	—	150	151
			Heart rate	—	63	60
8	60	25	FFA	0 56	0 50	0 48
			Glycerol	0 181	0 173	0 182
			Blood pressure	112	112	113
			Heart rate	61	61	62
9	60	25	FFA	—	0 73	0 75
			Glycerol	—	0 117	0 113
			Blood pressure	—	125	125
			Heart rate	—	60	60
10	60	25	FFA	—	0 53	0 59
			Glycerol	—	0 042	0 059
			Blood pressure	—	145	145
			Heart rate	—	68	68
11	60	25	FFA	—	0 35	0 43
			Glycerol	—	0 064	0 073
			Blood pressure	—	130	130
			Heart rate	—	68	72

in arterial blood plasma on systolic blood pressure (mm Hg) and on heart rate (beats min) in 11 men

During tilting			After tilting			
5—10 minutes	11—20	21—30	5 minutes	10	15	30
0 54	0 52	0 58	0 65	0 72	0 74	—
135	135	135	—	130	130	—
68	68	72	—	60	54	—
0 65	0 73	0 80	—	0 59	0 58	—
135	140	125	—	125	130	—
80	88	96	—	68	68	—
0 63	0 58	0 59	0 61	0 62	0 65	0 0
180	145	125	—	145	130	110
6	80	60	—	64		60
0 58	0 58	0 50	0 55	0 58	0 46	—
1 0	155	145	—	150	145	
63	68	72		64	64	—
0 47	0 59	—	0 58	0 65	0 61	0 56
175	135	—	130	130	120	115
80	84		72	72	68	70
	1 23	0 90		0 90		—
	0 170	0 117		0 096		—
	105	105		110	—	—
—	64	64		56	—	
0 39	0 44	0 60	0 63	0 51	0 57	
0 081	0 092	0 091	0 131	0 108	0 103	
174	174	161	154	159	—	
60	67	83	63	68	—	
0 44	0 52	0 53	0 57	0 64	0 53	
0 19	0 204	0 212	0 228	0 229	0 220	
125	127	118	115	113	113	
0	64	68	57	56	58	
—	0 85	0 76	—	0 89	—	0 89
—	0 129	0 127		0 131		0 132
—	120	115		115	—	120
—	100	97	—	78		0
—	0 77	0 81		0 79	—	0 78
—	0 047	0 059		0 050		0 058
—	125	130	—	130		115
—	80	80	—	64	—	67
—	0 50	0 48	—	0 50		0 50
—	0 064	0 070		0 077		0 077
—	90	95	—	120	—	120
—	76	68		[illegible]	—	0

Subjects with postural hypotension

One woman and five men with postural hypotension were studied. All the patients presented the typical picture of postural hypotension (3, 24) with a prompt fall in arterial pressure and no or only slight increase in heart rate on head-up tilting. Otherwise there were no important abnormalities as judged from the routine clinical and laboratory investigations. No glucose was found in the patients' urine.

Four of them (cases 1—4) had previously attended the Department of Endocrinology (Head: R. Luft), Karolinska Sjukhuset. Three of them (subjects 2—4) had previously been studied at the Laboratory of Clinical Physiology (Head: B. Jonsson), Karolinska sjukhuset.

Case reports

(Cases 2, 3 and 4 in this study correspond to cases 4, 1 and 2 respectively in the study by Bevegård et al. (3).)

Case 1. 48-year-old man with attacks of fainting since 1950. When he attended the Department of Endocrinology in 1955 his blood pressure was 160/105 mm Hg and the heart rate about 80 beats/min before head-up tilting. The urinary excretion of noradrenaline at rest was then 2.8 mμg/min. During 3 hours' tilting at 25° the blood pressure was about 80/55 mm Hg and the pulse rate did not change. The excretion of noradrenaline was 2.0 mμg/min and the excretion of adrenaline was not measurable. (Sundin (29) found that tilting of healthy subjects from recumbency to 75° induced a rise in the urinary noradrenaline secretion from 9.8 ± 1.4 at rest to 34.1 ± 3.7 mμg/min. The output of adrenaline increased from 1.9 ± 0.4 to 9.5 ± 1.3 mμg/min.) Before the present study the patient was generally unable to walk more than 20—50 m before he had to sit down to avoid fainting. The exercise test performed on a bicycle ergometer according to Sjöstrand (28) in the supine position showed that the maximal working capacity was markedly reduced: 250 kpm/min.

Case 2. 64-year-old man with typical postural hypotension for several years. At the time of the present study he was unable to stand in the erect position or to walk. He got extremely tired on sitting up in his bed for some minutes.

Case 3. 68-year-old man with symptoms of postural hypotension since 1954. The last year before the present study he could walk only 30—50 m before he had to sit down to avoid fainting.

Case 4. 68-year-old man. Postural hypotension for several years. Before the present study he had to resort to squatting because of fatique and dim vision after walking 10—30 m.

Case 5. 75-year-old female known to have hypertension from 1945. In the supine position the blood pressure was 220—200/110—100 mm Hg. From 1960 she had attacks of fainting. She attended the Department of Neurology, Serafimerlasarettet, 1961, where the diagnosis postural hypotension was established. At rest the urinary excretion of noradrenaline was 2.3 mμg/min and the excretion of adrenaline 1.8 mμg/min. Before and during insulin-induced hypoglycemia the noradrenaline excretion values were 4.4 mμg/min and 2.2 mμg/min respectively. The corresponding figures for adrenaline were 2.8 and 2.2 mμg/min respectively. The noradrenaline and adrenaline excretions were respectively 2.2 and 1.1 mμg/min before and 5.0 and 1.7 mμg/min after the administration of histamine.

At the time of the present study she was unable to walk more than 10—30 m. During an exercise test on a bicycle ergometer in the supine position there were ECG-changes suggestive of coronary insufficiency at 150 kpm/min.

Case 6. 60-year-old man since 1962 complaining of an increasing tendency to faint when standing or walking. He was admitted to the medical clinic in 1964, and soon after the

TABLE II Age blood pressure and fasting concentration of blood glucose and levels of cholesterol, phospholipids and triglycerides in plasma in 5 male and 1 female subjects with postural hypotension

Patient no	Age (yrs)	Blood pressure in supine position (mm Hg)		Blood glucose (mg/100 ml)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Triglycerides (mmoles/l)
		Systolic	Diastolic				
1	48	115	70	86	271	272	1 56
2	64	110	65	79	184	190	1 05
3	68	115	75	74	254	264	1 14
4	68	145	75	100	316	252	1 80
5 (female)	75	245	115	95	318	300	1 14
6	80	130	80	80	212	202	1 35

studies on lipid metabolism had been done he rapidly became mentally confused No further examinations were therefore performed

Animals

Three adult mongrel dogs were studied during head up tilting They were anaesthetized with Nembutal® (Abbott) 25—30 mg/kg i v with supplement when necessary

Methods

Tilting of subjects

No premedication was given before the metabolic studies which were performed in the morning after at least 15 hours fasting All drugs had been withdrawn several days before One teflon catheter was inserted into a brachial artery after local anaesthesia with prilocaine (Citanest® Astra) and one catheter into a brachial vein After at least 30 minutes rest in the supine position on a tilt table the subjects were tilted in the head up position for 20—30 minutes The tilting angle varied between 40 and 60 for the normal subjects The patients were tilted at an angle varying between 25 and 60 to produce a significant but tolerable blood pressure fall Some of the patients had to be tilted back before the end of the tilting period because of discomfort such as dizziness tiredness and nausea

Tilting of dogs

A teflon catheter was inserted into one brachial or femoral artery The dogs were tilted in the head up position during 30 minutes at an angle of 60

Analysis

Arterial blood was withdrawn into heparinized syringes No heparin was injected After immediate centrifugation plasma was extracted or precipitated promptly FFA were determined according to Dole s method (12) with the modification described by Trout et al (30) The turnover rate of FFA was studied in one of the patients with postural hypotension with the constant infusion technique (1 23) Labelled albumin bound palmitate was used as in previous work in this laboratory (2 9 21 22) with palmitic acid 9 10 H³ (New England Nuclear Co) as tracer The plasma FFA were isolated by thin layer chromatography and counted in a Packard Tri carb liquid scintillation spectrometer as described by Carlson et al (9)

Glycerol was estimated with the enzymatic method described by Wieland (32) The blood glucose levels were analyzed according to Marks (25)

TABLE III Effect of head up tilting on the concentrations of FFA (mmoles/l) and glycerol (mmoles/l) patients with postural hypotension

Patient no	Tilting angle	Tilting time		Before tilting 20 minutes	10	0
1	60	18	FFA	0 38	0 43	0 42
			Glycerol	0 107	0 128	0 099
			Blood pressure	115	125	135
			Heart rate	74	72	69
2	25	12 5	FFA	0 42	0 45	0 44
			Glycerol	0 055	–	0 070
			Blood pressure	110	110	110
			Heart rate	68	68	68
3	60	15	FFA	0 64	0 73	0 74
			Glycerol	0 093	0 099	0 108
			Blood pressure	115	110	110
			Heart rate	68	67	68
4	35	25	FFA	0 59	0 61	0 62
			Glycerol	0 102	0 097	0 103
			Blood pressure	145	140	130
			Heart rate	45	47	45
5	60	25	FFA	0 82	0 79	0 83
			Glycerol	0 146	0 112	0 116
			Blood pressure	246	240	240
			Heart rate	75	80	84
6	30	25	FFA	–	0 52	0 81
			Glycerol	–	0 055	0 322
			Blood pressure	–	130	130
			Heart rate	–	80	80

Concentrations of cholesterol phospholipids and triglycerides were measured several days prior to investigation as previously described from this laboratory (5 8)

All lipid and glucose determinations were made in duplicate Blood pressure was measured from the arterial catheter by means of an Elema Schonander pressure transducer (EMT 490 A) with its zero reference line always positioned at the same horizontal level as the heart In subjects 1—6 and 9—11 (table 1) and in two of the patients (cases 2 and 6) the blood pressure was measured over the brachial artery with a sphygmomanometer cuff in the usual manner the systolic radial pulse being felt for (to an accuracy of 5 mm Hg)

Results

FFA, glycerol, blood pressure and heart rate in normal subjects during head up tilting

There was a variable change in blood pressure readings during head up tilting (table I) The heart rate always increased The arterial levels of FFA before

in arterial blood plasma on systolic blood pressure (mm Hg) and on heart rate (beats/min) in 6

During tilting			After tilting		
5–10 minutes	11–20	21–25	5 minutes	10	15
0.49	0.59	–	0.70	0.73	0.74
0.119	0.166	–	0.183	0.166	0.145
61	59	–	138	125	107
70	69	–	70	70	70
0.39	0.42	–	0.65	0.52	0.47
–	0.083	–	0.102	–	0.074
65	55	–	140	130	120
68	68	–	68	68	68
0.67	0.70	–	0.86	0.98	0.95
0.103	0.114	–	0.135	0.143	0.130
71	68	–	111	120	131
74	76	–	70	69	68
0.71	0.82	0.87	1.02	1.14	1.00
0.113	0.146	0.163	0.180	0.181	0.145
71	75	55	108	121	130
45	45	45	45	45	44
0.71	0.75	0.78	1.05	1.16	1.20
0.111	0.135	0.154	0.199	0.183	0.157
137	126	101	167	191	238
81	80	77	71	75	80
0.92	0.91	0.80	0.84	0.83	0.75
–	0.242	0.245	0.253	–	–
70	70	70	140	135	130
82	82	82	82	82	82

tilting varied between 0.38 and 0.89 mmoles/l. The FFA concentration increased in all subjects during or after the tilting period. The mean rise, calculated from the lowest level before to the maximal level during or after tilting, was 0.22 mmoles/l. The increase calculated from the level immediately before to the level at the end of the tilting period varied between 0.01 and 0.29 mmoles/l.

The arterial levels of glycerol always went in parallel with the FFA levels (table I).

Patients with postural hypotension

The age, the blood pressure and the concentrations of blood glucose and plasma lipids in the patients are summarized in table II.

The head up tilting caused a prompt fall in blood pressure readings without

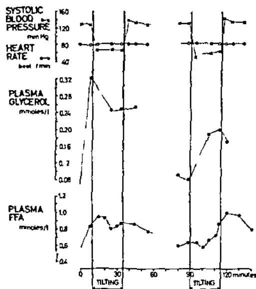


Fig 1 Concentrations of FFA and glycerol in arterial blood plasma blood pressure and heart rate in a patient (case 6 in table II) with disturbed sympathetic vasomotor control The subject was tilted in the head up position at an angle of 50 during two 25 minute periods

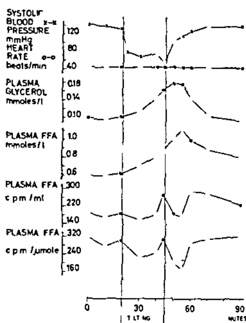


Fig 2 Concentration total activity and specific activity of plasma FFA concentration of glycerol in plasma blood pressure and heart rate in a patient (case 4 in table II) with disturbed sympathetic vasomotor control Palmitic acid —9 10 H³ was infused at a constant rate The subject was tilted in the head up position at an angle of 60 during one 25 minute period

or with almost no change in heart rate (table III) The arterial levels of FFA before tilting varied between 0 38 and 0 83 mmoles/l During or after the tilting the FFA levels increased in all subjects The mean increase calculated from the lowest level before to the maximal level during or after the tilting was 0 36 mmoles/l During tilting the FFA levels were seen to increase as well as decrease The changes calculated from the level immediately before to the level at the end of the tilting, varied between —0 17 and 0 17 mmoles/l

In one of the patients (subject 6) there was a marked rise of the FFA level, from 0 52 to 0 81 mmoles/l even before the tilting (fig 1) When this patient was tilted during a second period the FFA level did not start to increase until 10 minutes after the beginning of the tilting period The circulatory changes were similar during the two periods (fig 1)

In subject 4 the specific activity of plasma FFA decreased when the FFA concentration increased during and after the tilting period (fig 2) At the end of the tilting period there was a transient rise of the specific activity At the same time there was a further drop in blood pressure (fig 2)

The arterial levels of glycerol always increased together with the FFA levels (figs 1 and 2, table III)

Tilting of dogs

When the anaesthetized dogs were tilted from the supine to the head up position there was a fall in blood pressure readings and an increase in heart rate (fig 3) Immediately after the dogs had been restored to horizontal there was a transient rise of the blood pressure above the initial level No changes in plasma levels of FFA were seen (fig 3)

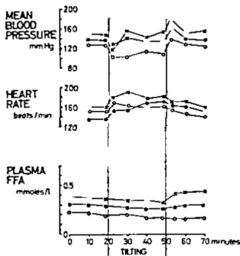


Fig 3 Concentrations of FFA in blood plasma blood pressure and heart rate in three anaesthetized dogs They were tilted in the head up position at an angle of 60 during one 30-minute period

Discussion

The sympathetic vasomotor activity is stimulated via baroreceptor reflexes during head up tilting (15 29, 31) Hamlin et al (17) previously reported that head up tilting, neuroadrenergic stimulation , also increased the plasma level of FFA in normal man, as was confirmed by the present study

Hamlin et al (17) studied a patient with autonomic insufficiency and suggested that the patient was unable to increase the plasma level of FFA during orthostatic stimulation Also Havel (19) reported that the FFA level in plasma did not increase in a patient with postural hypotension during head up tilting In contrast to these previous reports, the present study showed in all the patients with disturbed sympathetic cardiovascular control, FFA changes of the same type as those seen in normal individuals Hamlin et al (17), like Havel (19), and also Engelman et al (13) followed only the plasma concentrations of FFA in their patients with postural hypotension In this investigation the levels of glycerol in blood plasma were also followed, there being evidence that this glycerol level reflects lipolytic activity in adipose tissue under various conditions (6 7, 11 22) The plasma glycerol levels here always changed in parallel with the plasma levels of FFA, which suggests that the FFA changes were caused mainly by variations in the rate of lipolysis in adipose tissue The specific activity of plasma FFA decreased as the FFA concentration increased in a patient with postural hypotension which shows that the mobilization of FFA into blood plasma increased during tilting These findings indicate that in patients with postural hypotension head up tilting may induce a stimulation of FFA mobilization although there is no increased activity in sympathetic nerves of importance for cardiovascular control

In the anaesthetized dogs subjected to head up tilting external stimuli for FFA mobilization should have been

blocked due to the anaesthesia. No changes in the levels of FFA in plasma were seen. The results suggested that baroreceptor reflexes were stimulated in the dogs without increased mobilization of FFA. The studies on the patients with postural hypotension clearly demonstrated that the FFA rise was not correlated with the fall in blood pressure induced by head up tilting. It is therefore possible that the variable rise in plasma FFA concentration seen in man during head up tilting is caused not by the stimulation of baroreceptor reflexes but by the emotional stress induced by the different manipulations and the discomfort. The mechanism for the stimulatory effect on FFA in this instance is not known. However, it was recently demonstrated in anaesthetized dogs that electrical stimulation of sympathetic structures in the diencephalon and mesencephalon may selectively increase the blood pressure and heart rate or the plasma levels of FFA and glycerol (27). The effects were also seen in adrenalectomized animals. It is therefore possible that the stimulatory effect of orthostatic stimulation on the FFA mobilization is mediated by sympathetic nerves to adipose tissue.

Several findings indicate that the mobilization of FFA is of importance for the regulation of the concentration of plasma triglycerides and perhaps also of other lipids such as cholesterol (10). In the patients with postural hypotension studied here the concentrations of triglycerides and cholesterol were within normal ranges (5). These findings like the results of the experimental study indicate that the mobilization of FFA was not inhibited in these patients with impaired sympathetic vasomotor control. In the patient with postural hypotension previously studied by Havel (19) the plasma concentrations of triglycerides and cholesterol were abnormally low, 47 and 129 mg per 100 ml respectively, in spite of the fact that the patient had diabetes. The finding suggests that the patient represented another type of postural hypotension with impaired mobilization of FFA.

Summary

Eleven normal men were tilted in the head up position to induce an orthostatic stimulation of the baroreceptor reflexes. There was a variable increase in plasma levels of free fatty acids (FFA). The plasma levels of glycerol went parallel with the FFA levels. Six patients with disturbed sympathetic cardiovascular control (postural hypotension) were also studied during orthostatic stimulation. The changes in the levels of FFA and glycerol in the patients were similar to those in the normal individuals. The plasma concentrations of triglycerides and cholesterol in the patients with postural hypotension were within normal limits.

During tilting of three anaesthetized dogs the blood pressure decreased and the heart rate increased while the plasma FFA levels remained unchanged.

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The Effect of Estradiol and Progesterone on Plasma Lipids in Oophorectomized Women

By

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The lower incidence of coronary heart disease in women, especially in the fertile age has led many to believe that endocrine factors may be of importance in the pathogenesis prevention and also the treatment of atherosclerosis and its complications

How hormones could influence the metabolism of the vessel walls is not known The influence may be direct, or according to the filtration theory, secondary to changes in the blood composition

The metabolism of lipids must be involved in some way since great amounts of lipids are found in the arteriosclerotic plaques Furthermore the homeostasis of lipids in the blood is also often changed in patients with arteriosclerosis

Many studies have been devoted to the action of female sex hormones on blood and tissue lipids (reviewed by Cook (2) and Marshall (13)) However most studies in humans have been restricted to the level of cholesterol and total phospholipids and the patient materials have included post menopausal women old men patients with hypercholesterolemia or hyperlipemia and survivors of myocardial infarction

During pregnancy a pronounced change in lipid homeostasis occurs (27), and a characteristic finding is an alteration in composition within the phospholipid fraction in plasma The mechanism of these changes in lipid metabolism is not known but may be of hormonal origin The quantitatively most pronounced alterations in the hormonal production during pregnancy seem to concern estrogens and progesterone (8) The amount of these hormones produced during pregnancy increases considerably and is much greater than that administered to humans in earlier studies of the effect on blood lipids

In the present study the effects of estradiol and progesterone on the plasma level of cholesterol individual phospholipids triglycerides and free fatty acids have been determined in 3 other-

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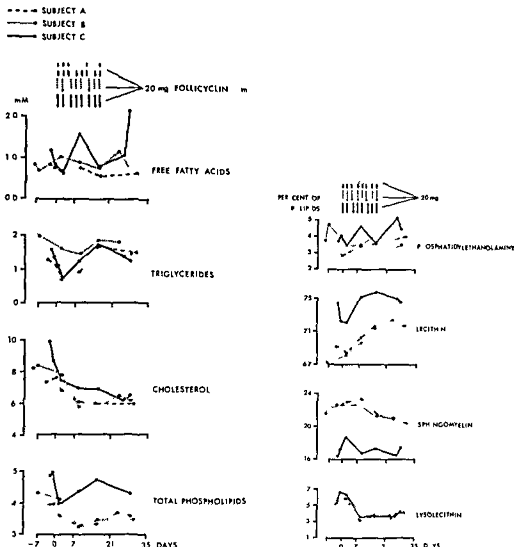


Fig 1 Plasma lipid fractions in 3 oophorectomized women after the administration of estradiol

wise healthy oophorectomized women The doses of the hormones were kept very high during treatment periods of 10—20 days

Material and methods

Three oophorectomized women were studied Subject A was 51 years old and had been operated 2 years previously on account of uterine myomas Subject B was aged 42 years and had been oophorectomized 8 years previously because of ovarian carcinoma. Subject C was 50 years old and had been oophorectomized 1 1/2 years earlier because of uterine myomas All the women were now healthy as judged by repeated clinical examinations They had taken hormonal substitution therapy only sporadically and not during the months preceding the study

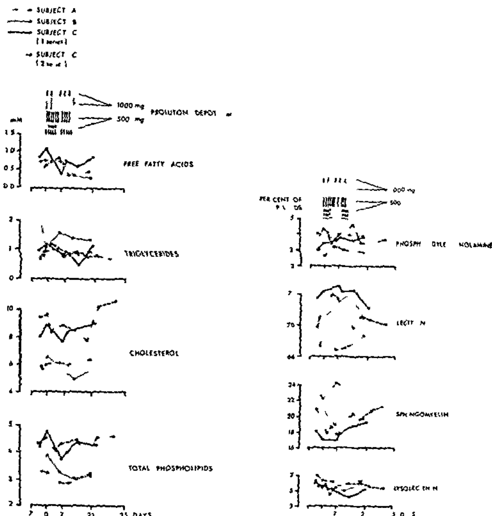


Fig 2 Plasma lipid fractions in 3 oophorectomized women after the administration of progesterone

Blood was obtained between 8 and 9 a m in the fasting state Citrated plasma was extracted and total phospholipids choles terol triglycerides and free fatty acids deter mined as previously described (25 26) For the determination of the phospholipid composition heparinized plasma was used The separation of individual phospholipids was achieved by thin layer chromatography (29)

Two or three control samples were taken before the injections were started Estradiol benzoate microcrystals (Folliclyclin Ciba) was injected i m in a dosage of 20 mg roughly every other day until 160 mg had been given over 2—3 weeks

17-α hydroxyprogesterone caproate (Proluton Depot Schering) was administered i m in a dosage of 500 mg every day or 1 000 mg every other day until 5 000 mg had

been given. At least two months passed between injection series. Each woman received one series of estradiol and one series of progesterone. Subject C received an additional series of progesterone injections in which however free fatty acids were not determined.

Results

The women tolerated the injections well and noted a subjective sense of well-being and an increased libido. After the estrogen injections a slight tenderness of the breasts was observed. Their appetite, body weight, and qualitative and quantitative food intake did not obviously change during the treatment periods.

The results of the lipid analyses are presented in figs. 1 and 2. The values of the individual phospholipids are presented as relative amounts of the total phospholipids.

After estradiol no evident trend could be seen with regard to free fatty acids or triglycerides. There was a distinct fall of cholesterol. Total phospholipids also showed a tendency to decrease. In the case of individual phospholipids there was an obvious decrease in the percentage of lysolecithin and increase in the percentage of lecithin.

After treatment with progesterone there were no obvious changes in any of the lipid fractions.

Discussion

In fertile women or in men administration of sex hormones may lead to alterations in the production and metabolism of the endogenous sex hormones. In order to minimize variations in the endogenous production of estrogens and progesterone during the investigation periods, oophorectomized women were chosen for this study. However, even in oophorectomized women injections of sex hormones may produce alterations in the function of other endocrine glands, and it seems difficult, if not impossible, to study the completely isolated effect of one hormone on blood lipids.

Only few reports have been published on the effect of estradiol on plasma lipids. Glass et al. (6) gave 0.25—0.75 mg estradiol buccally to 16 men and 15 women for 3—4 months and found no effect on the levels of cholesterol, phospholipids or total lipids. In addition 4 persons received a single i.m. injection of 5 mg estradiol dipropionate without effect. Russ et al. (22) gave a daily dose of 1.66 mg estradiol benzoate orally to one male patient and found a decrease of cholesterol and phospholipids. This patient also received among other medications para amino salicylic acid which now is known to decrease the plasma cholesterol level (19, 28). Oliver and Boyd (15, 16) gave 12 or 24 mg estradiol orally daily for 2 weeks to hypercholesterolemic men (3 subjects in each dosage group). They observed a slight fall in cholesterol of about 10 per cent and also a fall in the cholesterol/phospholipid ratio. As far as can be judged from the presented data the phospholipid level was hardly influenced. Furman et al. (5) administered 5 mg estradiol benzoate i.m. every other day for 24 days to a man with hypopituitarism and obtained a fall of about 10 per cent in both cholesterol and phospholipids. The effect of estradiol on triglycerides or on the

phospholipid composition has not been reported

In other studies of the estrogenic effect on blood lipids a variety of compounds have been used, especially the synthetic diethylstilbestrol the semi synthetic ethinyl estradiol and a preparation of conjugated equine estrogens A decrease of cholesterol was usually obtained, though in several cases especially with normal levels before treatment an increase was observed (1) The effect on plasma phospholipids has been reported to be even more variable Usually however, cholesterol has decreased more than phospholipids so that the cholesterol/phospholipid ratio has decreased (2, 13)

The present finding of a distinct fall in cholesterol and only a slight change in total phospholipids is thus in agreement with the majority of previous reports

The plasma triglycerides (calculated by difference) were reported to increase in eight male patients with various disease after ethinyl estradiol treatment (23) but Feldman et al (4) reported a decrease of triglycerides in patients with idiopathic hyperlipemia or hypercholesterolemia Recently Robinson and Lebeau (21) reported an increase of triglycerides after conjugated equine estrogens given to postmenopausal women in a dosage which lowered the levels of cholesterol but increased the phospholipids In the present study, in which a specific glyceride method was used there was however, no evident change in the triglyceride level

The plasma level of total phospholipids was reported by Jensen (9) to increase after treatment with diethylstilbestrol The increase was mainly in the alkali labile phospholipid fraction (mainly lecithin) while there was less or no increase in the alkali stable fraction (mainly sphingomyelin Other phospholipids were not measured Hagopian and Robinson 7 gave conjugated equine estrogens to postmenopausal women and found al o an i crease of total phospholipids This increase occurred in the lecithin fraction an observation which is in e ement with the present study where however n increase in the total phosp l pid level was observed In the pre t estigation a distinct decrease l sole ithin was observed and Hagop and R bin son also demonstrated a sim la trend

The effect of estrogens o fr e fa acids seems not to have been stud d in humans previously In rats Laron a d Kowadlo Silbergeld 11 administere daily doses of 5 or 10 mg estradi l benzoate for three days and obtained a significant increase This was a relatively much higher dose than used in the present study Due to day to day variations of the post absorbtive level of free fatty acids in humans (24) a larger material than the present one is necessary before definite conclusions can be drawn as to a possible influence of estradiol on the mechanisms which regulate the level of plasma free fatty acids

The effect of progesterone was studied by Engelberg and Glass (3) in 8 men and 7 women who received an oral daily dose of 10—30 mg for 3 –6 months and by Oliver and Boyd (16) in 6 hyper cholesterolemic men who received 100 mg daily i m for 5 days No change in

cholesterol or total phospholipids was obtained, in agreement with the present results, where also individual phospholipids, triglycerides and free fatty acids were uninfluenced by the hormone administration The present dose of progesterone was high and probably in the range of the amounts produced during pregnancy (8) It may, however, be noted that Zanetti and Tennent (30) obtained an increase of cholesterol after large doses of progesterone in dogs, and it cannot be excluded that large doses for a longer time (as in pregnancy) may increase the blood lipids even in humans

It seems more probable that estrogenic hormones may be involved in the production of the hyperlipemia of pregnancy, at least in the alteration of the phospholipid pattern which changed *in the same direction as in pregnancy* The increase in other lipid fractions in the hyperlipemia of pregnancy is more difficult to explain as an estrogenic effect but as noted above, alterations in blood lipids have been variable and it may be that a large amount of estrogens for a long time may have effects which are not easily reproduced experimentally

It is not known whether natural, semisynthetic and synthetic estrogens are similar in their effect on the blood lipids Robinson et al (20) found a relation between the dose of estrogen and the change in plasma cholesterol and total phospholipids Priest et al (18) observed that the relative response of different plasma lipids varied with different doses of estrogens Further studies on the dose response curves for different lipid fractions in humans are needed

The mechanisms by which estrogens change blood lipids are not known though recently Nestel et al (14) presented evidence to show that ethinyl estradiol hastens the turnover or catabolism of cholesterol

Whether the influence of estrogens on the plasma lipids is of importance for a proposed protection against atherosclerosis and allied vascular diseases remains to be established Oliver and Boyd (17) and Marmorston et al (12) found that in survivors of myocardial infarction ethinyl estradiol lowered plasma cholesterol but had no effect on mortality In contrast, conjugated equine estrogens had a significant effect on mortality in a dosage which had no effect on blood lipids (12) The obvious difference in the incidence of coronary heart disease *between men and women under 50* years of age (10) indicates that some sex hormones play an important role as protectors against these vascular diseases The observation that estrogenic hormones influence the homeostasis of different blood and tissue lipids emphasizes the importance of a more detailed knowledge of the mechanisms involved

Summary

The influence of large doses of estradiol and progesterone on plasma lipids was studied in 3 oophorectomized women

One hundred and sixty mg of estradiol benzoate administered i m within a period of 2—3 weeks caused a distinct fall of cholesterol but no change in triglycerides or free fatty acids Total phospholipids tended to fall but less than cholesterol Among the individual

phospholipids the percentage of lysolecithin fell and the percentage of lecithin rose

Five thousand mg of 17 α hydroxiprogesterone caproate administered i m within 10—12 days did not influence the plasma lipid level

Acknowledgement

The investigation was supported by a grant from Ollie and Elof Ericsson s Foundation

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Serum Creatine Phosphokinase Activity in Chronic Alcoholism, in Connection with Acute Alcohol Intoxication

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In recent years it has become evident that muscular complaints occur in chronic alcoholism (8, 10, 15) An "acute muscular syndrome' has been described (10, 15) Its symptoms were pain tenderness and oedema of the muscles following a period of high grade alcohol abuse The glutamic — oxaloacetic transaminase (GOT), glutamic — pyruvic transaminase (GPT) lactic dehydrogenase and aldolase activities in the serum revealed a pattern indicating necrosis in both liver and musculature Histological examination of muscle biopsy specimens showed degeneration and necrosis (15)

A chronic muscular syndrome in chronic alcoholism was described in 1964 (8) The syndrome was characterized by slowly progressing weakness in the legs and sometimes in the arms also The biopsy specimens from the muscles of these patients revealed small areas of atrophy and destruction Finally, minor signs of abnormalities were observed in muscle biopsy specimens from alcoholics without muscular symptoms (8)

Hence there is reason to suppose that subclinical necrosis in the musculature may occur frequently in chronic alcoholism In this investigation a specific muscular enzyme creatine phosphokinase (CPK), was assayed in the serum from 79 alcoholics in connection with acute alcoholic intoxication At the same time the serum activities of GOT and GPT were measured Creatine phosphokinase determinations were also made in a control material The results of this examination will be discussed in the present paper

Material

The material comprised 75 male and 4 female chronic alcoholics between the ages of 18 and 72 years In each case there was a history of *alcohol abuse extending over many years* and all the patients had been hospitalized or treated as outpatients for alcoholism All the patients had consumed large quantities

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TABLE II GOT GPT and CPK activity in 9 alcoholics with CPK activity above 30 units

Age	CPK	GOT	GPT
34	37	58	27
48	33	85	44
57	48	105	27
38	56	66	29
40	41	71	28
32	39	53	22
42	31	258	153
50	36	31	9
17	113	71	11

creatine phosphokinase activities were 4 42, 4 30 and 4 65 respectively (table I) Figures 1, 2 and 3 show the frequency distributions of the values in the above mentioned groups

In group 4 (hospital patients) the lowest value was 0 3 units and the highest value 4 1 units In this group the mean serum creatine phosphokinase activity was 1 29 units (table I) As shown in the frequency distribution graphs (figs 1, 2, 3, 4), the serum creatine phosphokinase activities in group 4 were lower than in groups 1 2 and 3

Alcoholics Among the alcoholics serum creatine phosphokinase activities were found to range between 2 1 and 113 units (fig 5) The mean serum creatine phosphokinase activity was 12 99 units In 72 subjects these activities exceeded the highest value in control group 4, 4 1 units In 30 alcoholics the creatine phosphokinase activities exceeded the highest activity in the controls (12 4 units) In 9 alcoholics the creatine phosphokinase values were above 30 units

In the group with creatine phosphokinase values exceeding 12 4 units, 14 subjects showed a rise in GOT combined with normal GPT values In the same group 6 subjects had raised values for both GOT and GPT, and in one subject there was a rise only in the GPT activity In the group with creatine phosphokinase values above 30 units, 6 subjects had raised GOT and normal GPT activities (table II)

In all, 52 alcoholics had GOT activities above the normal range Raised GPT activities were observed in 24 subjects In one case there was raised GPT and normal GOT activity

Statistics

The material was analyzed statistically as follows A total of 181 creatine phosphokinase determinations were made for 5 groups, containing 18, 15, 41, 28 and 79 persons respectively As shown in table I, these 5 groups differ in their mean values

These differences are statistically significant inasmuch as a random distribution of the 181 determinations into 5 groups of the same size as those in question, would very seldom give rise to such a large variance in group means as was actually obtained An F test with 4 and 177 degrees of freedom respectively, gave an F ratio of 20, which is statistically significant at the 0 1 per cent level

Similarly, the mean for the alcoholics is significantly different at the 0 1 per cent level from the mean for the groups 1, 2 and 3 combined (F = 44, df 1 and 149 respectively) In the same way the mean for the group of hospital patients was significantly smaller at the

0 1 per cent level than that for groups 1 2 and 3 combined ($F = 53$ df 1 and 98 respectively) On the other hand the means for the three groups 1, 2 and 3 do not differ significantly ($F = 0 2$ df 2 and 7 respectively)

In short statistical analysis of the data indicates distinct differences between the groups the main differences were found in the three groups hospital patients ambulant controls and alcoholics

Discussion

Several investigators have found increased serum creatine phosphokinase activity in patients with muscular dystrophy (4 5 20 21 24 26) polymyositis (20) dermatomyositis (20) and after acute myocardial infarction (4 7 20 21 29) In patients with neurogenic muscular atrophy only slight increase in serum creatine phosphokinase activity has occasionally been observed (20 22 24)

It has been stressed that muscular exercise is an important factor in producing increased serum creatine phosphokinase activity Strenuous muscular work has been stated to cause an increase in creatine phosphokinase aldolase and lactic dehydrogenase (2 14 27 30) Griffiths found that the upper normal limit of creatine phosphokinase activity for adult ambulant subjects was roughly twice that for nonambulant subjects (13) He concluded that prolongation rather than severity of muscular activity appears to be the important factor since the results of other investigators indicate that short term physical work has no effect on the serum creatine phosphokinase activity (23 25)

Herschkowitz et al found raised levels of creatine phosphokinase in the cerebrospinal fluid from patients with cerebral disorders whereas the serum creatine phosphokinase activity was normal (19) In a preliminary communication Acheson et al reported raised serum creatine phosphokinase values in 15 out of 24 patients with an acute cerebral vascular accident (1)

Raised serum creatine phosphokinase values have been recorded in hypothyroidism (9 11 12) Histological examination of muscle biopsy specimens from patients with myxoedema has revealed small abnormalities indicating muscular damage which may explain the raised serum creatine phosphokinase activities in patients with hypothyroidism (33)

Patients with liver diseases including acute viral hepatitis have been found to have normal serum creatine phosphokinase activity (4 20 24 29)

Nor does it seem likely that malignant disorders pernicious anaemia haemolytic anaemia or chronic renal disease affect the serum creatine phosphokinase activity (20)

Thus from the literature it appears probable that with the exception of acute cerebral vascular accidents a rise in serum creatine phosphokinase occurs only after disorders affecting skeletal or cardiac musculature and following muscular exercise

In control groups 1—3 the subjects had been working before samples were taken The creatine phosphokinase values in these groups were considerably higher than in group 4 which comprised hospital patients with low physical activity It

should be pointed out that the creatine phosphokinase values in group 4 are about the same as the normal values given by Pearce et al (25), and Duma et al (7) Pearce et al took their samples after 12 hours of rest in bed, and Duma et al used hospital patients as controls It is likely that muscular activity produced the higher creatine phosphokinase values in control groups 1, 2 and 3 However, in ambulant subjects Hughes obtained values about the same as those in group 4 (21) For his normal material Hughes employed outpatients attending for routine haematologic examination and healthy laboratory workers As blood samples are usually taken in the morning, it is probable that Hughes normal subjects" had not been working before the samples were drawn This may explain the difference between the creatine phosphokinase activity in Huges ambulant subjects and that in control groups 1, 2 and 3

The samples from the alcoholics were obtained after 2—24 hours of resting in bed Consequently it is difficult to decide which control group the alcoholics should be compared with Indeed, several samples from the material consisting of patients were taken from strictly ambulant subjects However some samples from the alcoholic material were taken after more than 12 hours of rest in bed As has already been pointed out, after their subjects had rested 12 hours in bed Pearce et al obtained normal values similar to those in control group 4 When compared with the highest value in group 4 (4 1), it is evident that 72 alcoholics had raised serum creatine phosphokinase activities On the other hand, compared with the highest creatine phosphokinase value in groups 1—3 (12 4), 30 alcoholics had raised serum creatine phosphokinase activities Nine alcoholics had creatine phosphokinase values above 30 units (table II) Thus, a considerable increase in serum creatine phosphokinase activity had occurred in some cases in the material consisting of alcoholics

No disorders, known to affect the serum creatine phosphokinase activity, were observed Thus, the raised serum creatine phosphokinase activities in the material consisting of alcoholics may be related to the patients' alcoholism However it is not possible to determine whether the observed, raised levels of sermu creatine phosphokinase have been due to skeletal muscular or cardiac muscular damage

It is not known whether the death of the cells must precede detactable creatine phosphokinase increases in the serum Normally, the cell membrane retains intracellular enzyme protein in high concentrations A variety of findings indicate that enzyme loss, as a result of cell membrane damage, can occur without the cells dying (6, 18, 31, 32) Accordingly, the observed rise in creatine phosphokinase in this material could be due to the effect of an increased permeability of the cell membrane without coexisting muscle cell necrosis

It is well known that in chronic alcoholism an appreciable increase in the activity of different liver enzymes occurs in connection with acute alcoholic intoxication In this material 24 subjects showed signs of acute liver damage in raised GPT activity Judging by the

creatine phosphokinase activities exceeding the highest control values, muscle damage occurred in a total of 30 alcoholics

In view of these findings it seems probable that muscular damage, in conjunction with acute alcoholic intoxication in chronic alcoholism, is as usual as liver damage However, after an alcoholic debauch the serum GOT activity often rises more rapidly than the serum GPT activity (16) In fact, a raised serum activity of a specific liver enzyme ornithine carbamyl transferase, has been recorded 6—11 days after a dose of alcohol given to healthy volunteers (3) Perhaps a muscle enzyme pattern appears earlier than a liver pattern after an alcoholic debauch, as has been found after barbituric acid poisoning (17) In this material only a single GOT and GPT determination was made If the enzyme determinations had been repeated for some days it is possible that in many cases a liver enzyme pattern would have appeared

The investigation has shown that muscular engagement in connection with acute alcohol intoxication, seems to be quite a common occurrence

Summary

Creatine phosphokinase activity (CPK) was determined in the serum from 79 alcoholics in connection with acute alcoholic intoxication It was found that 30 alcoholics had creatine phosphokinase values above the highest value in a control material Rather high creatine phosphokinase values (above 30 units) were observed in 9 subjects The highest creatine phosphokinase activity recorded was 113 units

The results indicate that muscular involvement is a common occurrence in chronic alcoholism

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From the Departments of Clinical Physiology, Medicine and Medical Biochemistry University of Goteborg Goteborg Sweden

Amino Acids and Free Fatty Acids in Plasma in Diabetes

II The Myocardial Arterio-venous Differences before and after Insulin

By

A Carlsten, B Hallgren, R Jagenburg, A Svanborg and L Werkö

The myocardial metabolism of oxygen and different substrates has earlier been studied in healthy individuals by the measuring of the myocardial arterio-venous differences (3, 5) Most substrates including glucose, fatty acids and lactic acid are extracted in proportion to their arterial levels (1) Among the individual free fatty acids there was a comparatively higher a v difference of oleic acid than of linoleic acid (5) The amino acids did not change during the myocardial passage except for an increase in alanine In the present investigation the myocardial a v differences have been studied in diabetics before and after insulin administration Some preliminary observations (4) indicated that there might be differences in the myocardial handling of individual free fatty acids between diabetics and healthy individuals

Materials and methods

Ten diabetics four women and six men aged 25—46 years with duration of the diabetes of 2—33 years were studied one of them on three occasions and one of them on two occasions On eight occasions analyses were made both before and thirty minutes after the intravenous administration of 8—20 IU of crystalline insulin The catheterizations were performed in the morning The patients had been fasting over night and no insulin had been given since the day before Seven of the patients were on an ordinary diet This diet included about 2 000 calories per day with 30—40 per cent of the caloric intake from fat 15—20 per cent from protein and with a high amount of starch rich vegetables but a restriction of bread milk potatoes and sweet food Three of them were on a carbohydrate rich diet earlier described (6) Keto-acidosis was not present and the Legal test was negative in the urine at the time of investigation The observations in the diabetics before insulin were compared with those in healthy controls including the individuals reported on earlier (3 5) one 27 years old woman and one 50 years old male

The effects of insulin in the diabetics were compared with those in a non-diabetic control group including three healthy male individuals aged 26 30 and 48 years two male patients with essential hereditary hypercholesterolemia aged 36 and 49 years and one male patient with hyperlipemia and xanthomatosis aged 38 years To hose sub-

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healthy controls, 116 and 126 ml/litre of blood respectively The a v-difference of glucose was also similar in both groups, 8 and 9 mg/100 ml of blood respectively, in spite of a higher arterial glucose concentration in the diabetics (331 mg/100 ml of blood) than in the controls (97 mg/100 ml of blood) The a v difference of lactate was 0 15 mM in the diabetics and 0 28 mM in the healthy controls at arterial levels of 0 9 mM in both groups The a v difference of pyruvate was 0 02 mM in both groups at arterial levels of 0 04 and 0 06 mM respectively

The arterial level of total free fatty acids (FFA) was as an average 1,279 μM in the diabetics and 693 μM in the healthy controls the decrease during the myocardial passage was 137 μM (11 4 per cent of the arterial level) and 133 μM (18 2 per cent) The arterio-venous differences of the individual fatty acids as a percentage of the arterial level were not uniform (table I) In both groups there was as a mean a higher percentage extraction of oleic acid (diabetics 12 5 per cent controls 27 9 per cent) than of linoleic acid (diabetics 8 5 per cent controls 10 6 per cent) resulting in a significant change in the percentage composition of the FFA fraction, the arterio venous difference of the percentage of oleic acid minus that of linoleic acid differed from zero both in the diabetics ($p < 0\ 05$) and in the healthy controls ($p < 0\ 001$)

The levels of the individual free amino acids (table II) were not significantly altered during the myocardial passage except for an increase in alanine in both groups ($p < 0\ 001$)

TABLE II The myocardial arterio-venous difference of amino acids before insulin
A = Arterial level mg/100 ml plasma
B = A v-difference mg/100 ml plasma

		Diabetics n = 11 M ± SEM	Healthy controls[1] n = 8 M ± SEM
Threonine	A	1 24 ± 0 081	1 88 ± 0 222
	B	01 ± 052	11 ± 052
Proline	A	1 77 ± 0 137	2 88 ± 0 272
	B	03 ± 059	07 ± 115
Glycine	A	1 35 ± 0 100	1 63 ± 0 086
	B	07 ± 049	07 ± 039
Alanine	A	1 67 ± 0 130	2 46 ± 0 272
	B	- 17 ± 035	43 ± 151
Valine	A	3 12 ± 0 222	2 56 ± 0 126
	B	01 ± 053	04 ± 048
Isoleucine	A	1 20 ± 0 091	0 90 ± 0 049
	B	01 ± 025	09 ± 039
Leucine	A	2 12 ± 0 146	1 49 ± 0 107
	B	03 ± 049	07 ± 064
Tyrosine	A	0 88 ± 0 046	1 00 ± 0 100
	B	07 ± 058	04 ± 073
Phenylalanine	A	0 79 ± 0 086	0 85 ± 0 077
	B	02 ± 065	04 ± 060

[1] This material includes the 7 individuals reported on earlier (3) and one male 50 years of age

The myocardial a v difference after insulin administration

The total body respiratory quotient changed after insulin in the diabetics from 0 72 ± 0 02 (M ± S E M) to 0 81 ± 0 01 and in the non-diabetic controls from 0 77 ± 0 05 to 0 87 ± 0 03

The myocardial oxygen extraction was not significantly influenced by the administration of insulin in the diabet

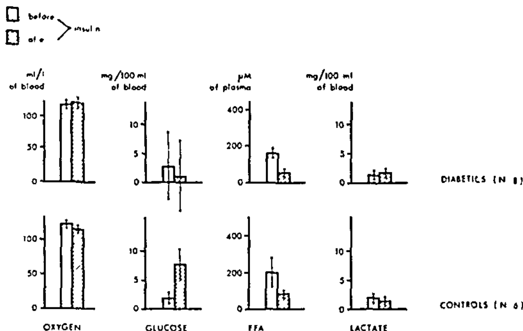


Fig 1 The myocardial arterio-venous differences of oxygen glucose FFA and lactate in diabetics and controls before and after insulin administration The heights of the columns for glucose FFA and lactate are proportional to the amount of oxygen needed for complete oxidation In the columns representing oxygen the amount needed for complete oxidation of the extracted quantities of glucose FFA and lactate is illustrated by dotted lines The bars show the standard errors of the mean

ics 118 ml litre before insulin compared to 117 ml litre of blood after insulin and in the non diabetic controls 128 and 119 ml litre respectively In the diabetics the arterial level of glucose decreased after insulin from 344 to 285 mg 100 ml of blood but no significant average myocardial uptake was observed 3 mg 100 ml before and 1 mg 100 ml of blood after insulin In the non diabetic controls the arterial glucose level decreased from 103 to 58 mg 100 ml of blood and the average myocardial uptake increased from 2 to 8 mg 100 ml of blood This difference in myocardial glucose extraction was however not statistically significant ($p < 0.2$) The results are summarized in fig 1

The arterial level of lactate averaged 1 0 mM before and 1 4 mM after insulin in the diabetics and 0 8 mM and 0 9 mM in the controls the a v differences averaged 0 1 mM and 0 2 mM in the diabetics and 0 2 mM and 0 2 mM in the controls The arterial level of pyruvate averaged 0 04 mM before and 0 07 mM after insulin in the diabetics and 0 05 mM and 0 08 mM in the controls the a v differences averaged 0 02 mM and 0 04 mM in the controls and no a v differences were observed in the diabetics

TABLE III The effect of insulin on the myocardial arterio-venous difference of free fatty acids A=Arterial level μM/l B=A v-difference μM/l C=A v difference per cent of the arterial level

		Diabetics n=8		Non-diabetics n=6	
		Before insulin M±SEM	After insulin M±SEM	Before insulin M±SEM	After insulin M±SEM
C_{l}	A	7 3± 1 9	2 1± 1 2	10 8± 2 8	8 0± 1 3
	B	-1 5± 0 8	1 4± 1 8	5 6± 2 9	2 2± 0 8
	C	-33 8± 21 3		2 7±28 0	26 1± 7 3
C_{14}	A	27 5± 2 8	9 1± 1 8	28 2± 5 8	11 8± 8 3
	B	0 3± 1 5	1 2± 1 6	7 5± 5 3	4 3± 1 0
	C	-1 1± 5 1	21 8±15 6	20 8±19 1	20 4± 4 2
C_{15}	A	5 7± 0 9	4 7± 1 0	8 6± 0 8	5 9± 0 5
	B	-1.9± 1 1	0 1± 0 8	0 8± 1 1	1 1± 1 6
	C	-42 8± 25 8	10 4±16 9	7 4±13 5	26 3± 35 8
$C_{16 0}$	A	254 5± 37 8	91 3±10 7	162 4±26 6	108 9± 12 6
	B	27 1± 11 4	3 3± 8 2	36 2±16 7	4 1± 8 4
	C	9 6± 3 5	3 8± 8 8	21 8± 9 0	7 1± 9 3
$C_{16 1}$	A	47 8± 4 6	15 1± 2 1	44 3± 5 4	27 0± 4 4
	B	11 0± 4 0	0 1± 0 9	14 1± 4 1	6 2± 3 5
	C	20 0± 6 9	4 6± 8 2	28 7± 6 4	16 9± 9 3
C_{17}	A	23 0± 2 4	8 6± 0 8	14 9± 1 8	11 1± 0 8
	B	3 1± 1 5	-0 2± 0 9	3 2± 1 1	0 1± 1 3
	C	12 6± 5 3	2 5±10 6	19 3± 6 2	1 6± 11 7
$C_{18 0}$	A	137 0± 24 2	61 7±10 6	78 5±14 3	61 1± 12 4
	B	14 3± 4 3	8 5± 3 4	17 0± 5 7	8 0± 2 2
	C	11 6± 2 6	13 1± 4 5	23 3± 9 6	16 9± 6 3
$C_{18 1}$	A	530 7± 40 9	162 8±15 2	430 3±25 4	221 2± 32 2
	B	83 3± 10 4	26 6± 6 8	109 3±20 0	57 2± 29 3
	C	14 1± 2 7	16 5± 4 1	31 9± 3 9	21 0± 9 2
C_{18}	A	184 3± 75 0	65 0±10 9	63 8± 3 7	43 9± 7 1
	B	15 3± 6 6	13 4± 6 4	11 8± 2 0	2 5± 6 3
	C	7 3± 3 4	16 5± 6 4	18 0± 2 4	0 3± 11 2
C_{19}	A	7 9± 1 8	4 4± 1 4	4 0± 0 5	3 5± 0 8
	B	2 7± 1 5	1 6± 1 5	0 8± 0 2	0 3± 0 5
	C	23 6± 10 4	22 0±23 4	21 7± 4 2	8 1± 17 4
$C_{0 1}$	A	38 6± 4 3	13 7± 1 5	20 2± 2 2	17 2± 2 5
	B	7 0± 2 3	0 1± 2 7	4 3± 0 9	2 7± 2 7
	C	15 9± 5 3	18 0±17 5	21 8± 4 1	11 3± 12 5
C_{0l}	A	9 3± 1 3	5 7± 2 3	8 0± 1 4	7 8± 1 6
	B	0 8± 0 8	1 5± 1 1	0 02± 0 8	1 2± 1 8
	C	-10 4± 8 6	48 7±47 0	3 5±10 2	-22 0± 20 1

Table III Cont

		Diabetics n = 8		Non-diabetics n = 6	
		Before insulin M ± SEM	After insulin M ± SEM	Before insulin M ± SEM	After insulin M ± SEM
C_{m}	A	17 4± 3 2	13 0± 2 2	11 5± 1 6	11 6± 2 0
	B	3 7± 0 6	3 1± 2 1	−0 6± 0 6	−2 8± 2 0
	C	22 7± 3 2	18 3±14 0	−5 6± 5 2	−17 6± 18 1
$C_{r p}$	A	8 6± 1 8	5 0± 0 8	8 1± 1 9	7 8± 1 6
	B	−1 1± 1 5	−0 4± 1 2	−0 6± 0 7	−1 6± 1 7
	C	−18 4± 21 2	−0 6±22 0	−23 5±15 5	−32 9± 12 7
Total FFA	A	1289 ±132 7	454 ±41 5	790 ±71 6	546 0±127 6
	B	158 ± 23 9	52 ±16 3	207 ±80 1	80 0± 16 5
	C	12 4± 1 7	11 8± 4 0	25 2± 5 0	13 4± 8 4

TABLE IV The effect of insulin on the myocardial arterio-venous difference of amino acids
A = Arterial level mg/100 ml plasma B = A-V-difference, mg/100 ml plasma

		Diabetics n = 3		Non-diabetics n = 3	
		Before insulin	After insulin	Before insulin	After insulin
Threonine	A	1 52	1 19	2 36	1 48
	B	19	− 03	33	13
Proline	A	2 16	1 74	2 63	2 14
	B	18	− 17	− 04	06
Glycine	A	1 52	1 28	1 85	1 42
	B	03	02	10	− 03
Alanine	A	1 71	1 76	1 97	1 87
	B	− 15	− 28	− 02	− 08
Valine	A	3 39	2 94	2 99	2 59
	B	04	09	13	− 21
Isoleucine	A	1 22	0 89	0 90	0 72
	B	07	01	− 01	07
Leucine	A	2 22	1 58	1 73	1 30
	B	19	10	13	28
Tyrosine	A	0 90	0 66	0 80	0 83
	B	− 10	− 03	− 24	26
Phenylalanine	A	0 84	0 70	0 86	0 67
	B	− 16	05	− 32	06

The arterial level of total FFA decreased after insulin in the diabetics from 1,289 μM to 454 μM and the myocardial extraction from 158 μM (12 4 per cent of the arterial level) to 52 μM (11 8 per cent) (table III) In the non diabetic controls the arterial level of total FFA was 790 μM before insulin and decreased to 546 μM after insulin The myocardial a v -difference of total FFA decreased from 207 μM (25 2 per cent of the arterial level) to 80 μM (13 4 per cent) Among the individual fatty acids the a v difference decreased in a similar way in both groups with the exception of linoleic acid, the extraction of which increased after insulin in the diabetics in 6 of 8 investigations A similar change was observed only in one case among the non diabetic controls

The myocardial a v differences of free amino acids were not influenced by insulin (table IV)

Discussion

A comparison of the myocardial extraction figures in the total group of diabetics before insulin administration with those in the healthy individuals shows no major difference The myocardial a v difference of oxygen lactate and pyruvate was similar and that was also the case regarding the a v difference of glucose and FFA although the arterial levels of these substrates were higher in the diabetics than in the controls

The total body respiratory quotient (RQ) increased after insulin both in diabetics and in the non-diabetic controls indicating a stimulation of the carbohydrate oxidation In the non-diabetics insulin increased the myocardial uptake of glucose in 5 of 6 individuals and lowered the FFA uptake in the same 5 cases In the diabetics insulin lowered the a v -difference of FFA in all the patients but did not influence the average myocardial a-v -difference of glucose which was low both before and after insulin The observed a-v differences of the substrates before insulin administration can be calculated to comprise about 65 per cent of the a v difference of oxygen both in diabetics and controls (fig 1) After insulin this figure increased to about 80 per cent in the controls but decreased to about 30 per cent in the diabetics This indicates that the diabetic heart muscle after insulin administration increases the oxidation of other substrates than those analysed here, e g ketone bodies plasma triglycerides or substrates stored within the heart muscle Butterfield and collaborators (2) have observed that not even in peripheral muscles is the uptake of glucose always stimulated by insulin in diabetes of juvenile type

When calculated as a percentage of the arterial level the myocardial extraction of FFA was similar before and after insulin in the diabetics (12 4 and 11 8 per cent) but decreased in the non diabetics from 25 2 per cent before to 13 4 per cent after insulin The arterial level of FFA was similar after insulin in the two materials (454 μM in the diabetics and 546 μM in the non diabetic controls)

In the diabetics the uptake of oleic acid was proportionally higher than the uptake of linoleic acid an observation which has earlier been made in healthy

controls also (3, 5). It is interesting to note that this difference in the myocardial handling of these fatty acids still existed although the diabetics due to nutritional circumstances generally had a higher percentage of linoleic acid in the FFA fraction.

In a previous study on healthy individuals (5) the arterial concentration of the C_{16} and C_{18} fatty acids were plotted against the coronary sinus concentrations. The figures indicated a linear relationship between these two variables and threshold levels below which no fatty acids were extracted by the myocardium. Due to the great spreading of the figures both at the high arterial levels before insulin and at the lower levels after insulin the present material does not allow any conclusion concerning arterial thresholds in the diabetics.

The difference in the net myocardial uptake of oleic and linoleic acid in relation to the arterial levels of these fatty acids observed in healthy individuals could be explained by different arterio-myocardial thresholds (5). The observation that the myocardial a-v difference of linoleic acid increased after insulin in 6 of 8 diabetics but decreased in 5 of the 6 non-diabetics indicates that the diabetic state influences the metabolism of this fatty acid. Nestel (7) recently emphasized an inverse relationship between the plasma level of triglycerides and the turnover rate of linoleic acid. There is a close correlation between the metabolism of glucose and triglycerides in the adipose tissue, in the liver and presumably also in the muscle. The variations in the turnover rate of the individual plasma FFA may thus be related also to the intensity of the glucose oxidation in the various tissues. If this is the case, the difference in the uptake of linoleic acid observed after insulin administration between the diabetics and the controls may be due to differences in the intensity of carbohydrate metabolism in the myocardium.

Summary

The myocardial arterio-venous differences of oxygen, glucose, lactate, pyruvate, individual free fatty acids and free amino acids were compared in diabetics and controls.

Before insulin administration there were no significant differences between the two groups in the myocardial extraction of these substrates. The extraction of glucose and free fatty acids was in the same order of magnitude in spite of the higher arterial levels in the diabetics.

Insulin administration did not increase the average myocardial glucose extraction in the diabetics and the blood glucose level was still enhanced. In the non-diabetic controls insulin increased the glucose extraction in all cases except one and the blood glucose level was markedly lowered.

Insulin lowered the myocardial free fatty acid extraction simultaneously with a lowering of the arterial free fatty acid concentration both in diabetics and controls. The arterial free fatty acid concentration was normalized in the diabetics.

The extraction of the individual free fatty acids decreased in both diabetics and controls in a similar way as the total free fatty acid fractions. However,

the extraction of linoleic acid increased after insulin in 6 of 8 investigations in the diabetics but in only one of the controls

Acknowledgement

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The 9th International Congress of Internal Medicine will be held in Amsterdam, September 7—10, 1966

President of the Congress Professor J G G Borst

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Main subjects of the congress Integration in Internal Medicine, Auto-immune diseases and Transplantation, Use of Corticosteroids in Internal Medicine, Radioisotopes in diagnosis and treatment Recent developments in Internal Medicine

Correspondence can be directed to Secretariat of the 9th International Congress of Internal Medicine c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands

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An *International Symposium on Atherosclerosis and Reticulo-endothelial System* will be held in Como Italy, on September 8—10, 1966 The Symposium is organized by the International Society of Reticulo-endothelial System, the European Society for Biochemical Pharmacology and the Società Italiana per lo Studio dell Arteriosclerosi

Secretary Prof Rodolfo Paoletti, Institute of Pharmacology, Via Vanvitelli 32, Milan Italy

Deadline for submission of papers May 31

The Rumanian Congress of Gastroenterology — with international participation — will be held in Bucharest, September 1967 The transactions lasting 3 days will comprise panel discussion and communications on the following topics chronic hepatitis gastric ulcers and ulcerative colitis Simultaneous translation into English, French German and Russian

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From the Department of Clinical Physiology, Glostrup Hospital Copenhagen Denmark

Renal Cortical Blood Flow and Split Function Test in Patients with Hypertension and Renal Artery Stenosis

By

J Ladefoged

Lesions of the renal artery may cause hypertension in humans It has been assumed that this might be due to a reduced blood flow to the kidney tissue The present study has been made to find out whether the renal blood flow is reduced in hypertensive patients with stenosis of the renal artery Furthermore investigation has been made into what extent an abnormal split function test can be combined with a low renal blood flow in kidneys with stenosis of the artery

Material

The study comprises 31 patients with a diastolic hypertension fulfilling the criteria of Master et al (13) In all the patients renal angiography showed stenosis of one or both renal arteries At the time of investigation the patients were aged 16 to 65 years As renal angiography was generally undertaken in all younger patients with diastolic hypertension the patients were unselected according to sex family history and severity and duration of the hypertension Patients with hypertension of known hormonal or cardiac aetiology were not included

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No normal subjects have been examined The control values were taken from the unaffected kidneys in 5 patients with normal p-aminohippuric acid clearance and slight hypertension

Methods

The patients were submitted to all the usual investigations for evaluation of a hypertensive patient including iodine 131 hippuran renography and intravenous urography To determine the significance of the stenosis demonstrated by the angiography a split function test (Howard or Stamey test was performed in nearly all patients The criteria proposed by Connor et al (4) Birchall et al (2) and Stamey et al (15) have been followed in the interpretation of the function study The opthalmoscopic findings were described as proposed by Keith et al 6) Clearance of inulin was measured with the technique described by H W Smith 14 Inulin in urine and plasma was determined by the method described by Bojesen 3)

The determination of the divided renal blood flow with the xenon 133 wash out technique (7 10) was carried out as follows the renal artery was catheterized from the femoral artery by the Seldinger technique After catheterization 1 to 5 mC xenon 133

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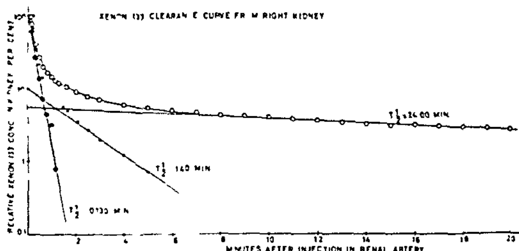


Fig. 1. Graphical analysis of a wash-out curve of xenon 133 from right kidney. The original curve is plotted against time on semilogarithmic paper and resolved in three compartments by the "peeling-off" method. The straight lines represent the resultant exponentials.

Table I. Clinical data and renal cortical blood flow in 8 hypertensive patients with renal artery

Case no.	Age yrs	Sex	Blood pressure (mm Hg)	Duration of hypertension (yrs)	Optic fundi	Excretory urography	Iodine 131 hippuran renography	Inulin clearance (ml/min/1.73 m²)
31	[illegible]	[illegible]	220 110	[illegible]	II–III	—	Abnormal on R	91
51	41	[illegible]	170 110	[illegible]	II	—	Abnormal on R	—
59	[illegible]	[illegible]	220 110	[illegible]	I–II	—	Bilat abnormal	–
62	[illegible]		160 110	15	II	Normal	Abnormal on R	–
67	46		200 110	[illegible]	I–II	Normal	Abnormal on R	103
75	58		180 115	[illegible]	III	—	Bilat abnormal	73
77	22	[illegible]	175/135	1	I	Normal	Normal	[illegible]
90	43	[illegible]	180 120	[illegible] (?)	II	Normal	Bilat abnormal	85

dissolved in sterile saline were injected rapidly into the renal artery The catheter was withdrawn immediately after the injection to the aorta below the renal artery to avoid disturbance of the renal blood flow The wash out curve of the radioactive gas from the kidney was measured with an externally placed scintillation detector coupled to a rate meter (time constant 1 second) and to a linear recorder If there was more than one artery to a kidney the measurement was carried out on each branch The measurement was performed on both kidneys

The formula for calculating the cortical blood flow from the desaturation curves is based on the proposals of Kety (8) According to his approach the amount of inert gas in the tissue during desaturation is given by a series of exponential functions

$$Q_t = \sum_{i=1}^{n} Q_{io} e^{-k_i t}$$

where

Q_t = amount of gas in a tissue at time t

Q_{io} = amount of gas in a tissue at time t = o

The clearance constant $k_i = \frac{F_i \ \delta_i}{W_i \ \lambda}$ where

F_i = blood flow in ml/min

W_i = weight of a tissue

δ_i = specific gravity of a tissue

λ = partition coefficient tissue blood

In fig 1 is shown a conventional graphical analysis of a curve assuming three such exponential terms Form the clearance constant k_1 of the first (fastest) component the renal cortical blood flow CRBF was calculated as

$$CRBF = F_1/W_1 = \frac{k_1 \ \lambda}{\delta_1}$$

The specific gravity of kidney tissue was taken to be one

From in vivo rat experiments λ was found to 0 7 at haematocrit 50 per cent (Andersen

stenosis and abnormal split function test

Localisation and grade of stenosis on aortogram	Relative size of the kidneys	Side of abnormal split function test	Renal cortical blood flow (ml/g min) R	L	Significance of difference(s) in blood flow (1 % level)	
R (severe) L (slight)	R<L	R	2 2 2 5	2 9 3 0		s
R (moderate)	R<L	R	2 3	3 3		s
R (severe) L (severe)	R>L	L	1 2	1 8		n s
R aber (moderate)	R=L	R	Main 2 7	2 4	$R_{main} \sim R_{aber}$	s
			Aber 2 0		$R_{main} \sim L$	n s
L main (severe) R (slight)	R=L	L	3 0	Main 2 5		n s
L aber (moderate)	R=L	L	4 1	Main 3 0	$R \sim L_{main}$	s
				Aber 1 5 1 3	$L_{main} \sim L_{aber}$	s
L (occl of lower branch)	R<L	L	6 3	3 8 3 8		s
R (severe)	R<L	R	2 2 2 3	2 2 2 4		n s

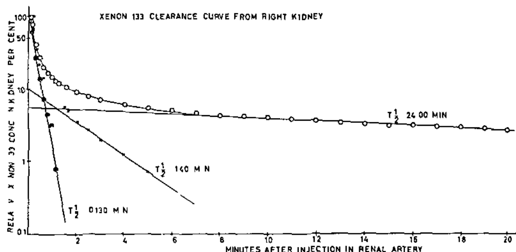


Fig 1 Graphical analysis of a wash out curve of xenon 133 from right kidney The original curve is plotted against time on semilogarithmic paper and resolved in three compartments by the peeling off method The straight lines represent the resultant exponentials

TABLE I Clinical data and renal cortical blood flow in 8 hypertensive patients with renal artery

Case no	Age (yrs)	Sex	Blood pressure (mm Hg)	Duration of hypertension (yrs)	Optic fundi	Excretory urography	Iodine 131 hippuran renography	Inulin clearance (ml/min/1 73 m²)
31	55	♂	220 110	$^1/_2$	II—III	—	Abnormal on R	94
51	41	♂	170/110	1	II	—	Abnormal on R	—
59	65	♂	220/110	$^1/_2$	I—II	—	Bilat abnormal	—
62	51	♀	160/110	15	II	Normal	Abnormal on R	—
67	46	♀	200/110	$^1/_2$	I—II	Normal	Abnormal on R	103
75	58	♀	180/115	$1^1/_2$	III	—	Bilat abnormal	73
77	22	♂	175/135	1	I	Normal	Normal	96
90	43	♂	180/120	$^1/_2$ (?)	II	Normal	Bilat abnormal	85

stenosis and normal split function test

Inulin clearance (ml/min/1 73 m²)	Localisation and grade of stenosis on aortogram	Relative size of the kidneys	Renal cortical blood flow (ml/g min)		Significance of difference(s) in blood flow (1 % level)	
			R	L		
—	R (slight)	R=L	2 8 2 7	3 2-3 5		s
—	Bilat (slight) 2 arteries to each kidney all affected	R<L	Main 2 3 1 9 Aber —	Main 2 2 Aber 2 1		n s
—	L (slight)	R=L	2 3	2 1 2 3		n.s
46	R (moderate)	R≪L	3 8 3 6	2 4		s
—	L upper (slight) R (fibromuscular hyper plasia)	R=L	2 3	Upper 2 7 Lower 2 8		n.s
116	L aber (slight)	R=L	3 3	Main 3 1 Aber 1 8	$L_{main} \sim L_{abe}$	s
—	L inferior branch (slight)	R=L	3 5 2 8	3 1-2 7		n.s
—	L (slight)	R>L	3 3	2 4		s.
97	R (slight)	R=L	3 1	Upper 3 3 Lower 2 6	$R \sim L_{up}$ $L_{up} \sim L_{low}$	n.s s
116	L (slight)	R=L	3 9	4 0		n.s

Results

The control values for renal cortical blood flow varied from 3 6 to 6 3 ml/g min

In the first part of tables I II and III the most important clinical data such as blood pressure duration of hypertension description of optic fundi, results of excretory urography, iodine 131 hippuran renography and inulin clearance are given for the 31 patients

Eight of the patients had an abnormal split function test The stenosis was "severe and moderate never' slight From table I appears that the cortical blood flow to the kidneys with renal artery stenosis varied from severely reduced (no 59) to normal (no 77), but reduced values prevailed Significant differences in the cortical blood flow between the stenotic and the non stenotic kidney were present in four cases (nos 31, 51, 75 and 77) Only two of the patients (nos 75 and 77) had a normal blood flow to the non stenotic kidney

A normal split function test was found in ten cases Table II gives the findings in these patients They all had slight stenosis of the artery The blood flow to the stenotic kidney varied as in group I within a wide range Significant differences between stenotic and non stenotic kidneys were found in four cases

TABLE II Clinical data and renal cortical blood flow in 10 hypertensive patients with renal artery

Case no	Age (yrs)	Sex	Blood pressure (mmHg)	Duration of hypertension (yrs)	Optic fundi	Excretory urography	Iodine 131 hippuran renography
IV	55	♀	150/110	8	I—II	Abn on R	—
4	38	♂	150/100	<½	I	Normal	—
19	59	♂	180/120	5	I—II	Normal	—
39	43	♀	160/120—130/80	10	I—II	R kidney small and poorly excreting	Abn no R
43	55	♀	170/100	10	II	Normal	—
47	64	♀	180/120	?	III	—	—
53	61	♂	200/110	<1	I	Normal (small concrement in left kidney)	—
58	45	♂	140/110	½ (?)	IV—II	Normal	Abn on L
64	46	♂	230/130—170/100	½	II—III	Normal	Abn on R
89	17	♂	190/120	3 (?)	II	Normal	Normal

and Ladefoged 1965) Correction for haematocrit was according to the formula

$$\lambda Hct_x = \lambda Hct_{44} \frac{1\ 69}{1\ 05 + 0\ 013\ \ Hct_x}$$

Experimental accuracy

From 21 repeated measurements it was found that a difference between two blood flow figures is significant if the difference exceeds 0 7 ml g min (99 per cent confidence limit) If duplicate measurement is carried out in each kidney the difference between the two mean values is significant if it exceeds 0 5 ml/g min (99 per cent confidence limit) If duplicate measurements are performed in one kidney and a single measurement in the other the limit for significance is 0 6 ml/g min

Roentgenological considerations

The series comprises patients with all degrees of stenosis of the renal artery including minor lesions of doubtful significance The stenosis has been graded as slight moderate and severe Slight' if the diameter of the artery measured on the X ray film at the point of maximal narrowing was more than 40 per cent of the diameter proximal to the stenosis, moderate if the stenosis constricts the artery to about 50 per cent (40 % to 60 %) and severe' stenosis with less than 40 per cent of the original diameter This grading is based on the system suggested by Sutton et al (16) The last two groups include all the cases in which there was a poststenotic dilatation of the artery

The relative size of the kidneys was determined by planimetry of the nephrographic phase of the renal angiography A difference in the areas between the two kidneys of more than 10 cm² on the conventional roentgenogram with a film to focus distance of one meter was considered significant

stenosis The split function test was not carried out or the performance was unsatisfactory

Inulin clearance (ml/min/1 73 m²)	Localisation and grade of stenosis on aortogram	Relative size of the kidneys	Renal cortical blood flow (ml/g min)		Significance of difference(s) in blood flow (1 ° level)	
			R	L		
61	L (moderate)	R⩾L	2 5 2 7	3 2		s
6[illegible]	L (moderate)	R⩽L	2 3	2 4		n s
—	R (slight)	R⩽L	1 8	3 8		s
150	R (slight)	R<L	4 2	4 2		n s
122	R (slight)	R=L	2 9 3 2	3 2		n s
—	L (slight)	R=L	2 5 3 0	3 0 3 0		n s
110	R (moderate)	R=L	2 6	3 2 3 2		s
—	L (severe)	R⩾L	2 5 2 3	4 0		s
81	R (moderate)	R=L	2 5 2 6	3 3 3 1		s
52	L aber (slight)	R<L	3 1	Main 2 9 3 0	L_m in L_{aber}	s
				Aber 1 9		
104	R upper (slight)	R<L	Upper 3 9	Upper 4 2		n s
			Lower 4 2	Lower 3 8		
128	L (slight)	R=L	3 1 2 8	3 1 3 4		n s
127	L lower (slight)	R=L	3 6 3 8	Upper 3 1	$R \sim L_{upper}$	s
				Central 4 0	$R \sim L_{central}$	n s
				Lower 3 4	$R \sim L_{low}$	n s

normal arteries and to renal tissue supplied by a stenosed artery were found in three cases (nos 66, 71 and 73)

Relationship between renal cortical blood flow and results of the split function test

Table IV summarizes the data from tables I and II In the group with abnormal split function test four of eight patients had a significant difference between the renal cortical blood flow to the stenotic and to the non stenotic kidney A significant difference was present in four of ten in the group with normal split function test This difference — four out of eight versus four of ten — between the two groups is not statistically significant

The mean values of the blood flow to the stenotic kidneys in the two groups were nearly the same 2 5 and 2 8 ml/g min, respectively, the same figures for the non stenotic kidney in the two groups were 3 2 and 3 0 ml/g min The

TABLE III Clinical data and renal cortical blood flow in 13 hypertensive patients with renal artery

Case no	Age (yrs)	Sex	Blood pressure (mmHg)	Duration of hypertension (yrs)	Optic fundi	Excretory urography	Iodine 131 hippuran renography
16	50	♀	200/110	>5	I—II	Poor excretion on L	—
40	54	♂	210/130	1/2	I—II	No excretion on R	No uptake on R Abn on L
41	43	♀	220/150—170/90	4	II	R kidney hypoplastic	—
44	41	[illegible]	150/110—170/90	[illegible]	0	Normal	—
45	16	♀	220/120	[illegible]/2	II	Normal	Abn on R
56	62	♂	170/110	[illegible]	I	Normal	Normal
66	65	♂	200/1[illegible]	>4	I	Normal	Normal
68	61	[illegible]	190/1[illegible]	3	—	L kidney small with normal excretion	—
71	54	♀	180/10[illegible]	2	I	Normal	Normal
73	55	♂	[illegible]00/11[illegible]	[illegible]	I—II	—	Bilat abn
81	43	[illegible]	190/11[illegible]	4 [illegible]	I	—	Normal
83	49	[illegible]	2[illegible]/[illegible]0	[illegible]	III	Normal	Normal
84	41	[illegible]	180/110		I	Slight decreased density of contrast in pelvis on R	Normal

(nos [illegible] 39 4[illegible] [illegible]) in one of them (no 39 the hi[illegible] [illegible]ure was found in the stenotic kid[illegible]

Split function [illegible] was not carried out in seven patient[illegible] [illegible]n another six the performance was [illegible] unsatisfactory that interpretation was not possible Table III gives the data from these patients It includes cases with all degrees of stenosis of the artery In three of them the stenotic kidney was much smaller than the other (nos 16 41 and 68) Two of these small kidneys had a higher blood flow than the contralateral non stenotic kidney (nos 16 and 68) One patient (no 40) had stenosis of one kidney and the other was quite small, but there was no significant difference in the blood flow between the two sides

The rest of table III gives data from nine patients with slight or moderate stenosis of only one of the renal arteries Significant differences in renal cortical blood flow to renal tissue supplied by

are expressed in ml per tissue weight per minute, whereas the usually employed para aminohippuric acid clearance method gives the total blood flow per minute through the kidney This difference can have some advantage in studies of renal ischaemia , since the xenon 133 wash out method reveals variations in the blood flow not proportional to variations in the kidney weight

Goldblatt and co workers (5) have assumed that ischaemia of the renal tissue is the initiating factor in the production of the renal hypertension It has, however, not yet been possible to confirm this assumption by animal experiments or by observations in patients

In the present material all but one (no 77) of the patients with an abnormal split function test had reduced blood flow per gram tissue in the stenotic kidney The reduction in blood flow to renal tissue supplied by a stenotic artery was on average about 25 per cent This figure is in agreement with the results of Maier et al (12) in experimental stenosis in dogs

However, the split function test was abnormal in some patients without a difference in the blood flow between the stenotic and the non stenotic kidney An abnormal split function test is therefore evidently not dependent on a difference in blood flow per gram tissue between the two kidneys

Reduced cortical blood flow and difference in the blood flow between the stenotic and the non stenotic kidney were also found in patients in whom other investigations not were suggestive of a renal genesis of the hypertension In some of these (nos 47 73 and 84) the reduced blood flow was found in a stenotic artery, which only supplied a smaller segment of the kidney Demonstration of a reduced blood flow in a part of a kidney may indicate a renal genesis of the hypertension in spite of a normal split function test as that method often fails in demonstration of segmental stenosis

However, three patients (nos 43, 58 and IV) with stenosis of the main renal artery had reduced blood flow to the stenotic kidney compared with the blood flow to the non stenotic, but a normal split function test From the present investigations in these three patients it is difficult to conclude anything about the significance of this blood flow reduction It may be due to unilateral kidney disease coincident with but not responsible for the hypertension

The group of patients in table II with stenosis without differences in the blood flow between the two kidneys and normal split function test are probably cases of essential hypertension with coincident stenosis Several investigations in the last years have given evidence for the existence of such stenosis in many patients with or without hypertension (9 11) Some of the patients in table III may belong to the same category (nos 44, 45 56 81 and 83) but a final classification is not possible here without a split function test

The occasional finding of a normal blood flow to the stenotic kidney but reduced blood flow (per gram) in the normal one (nos 16 and 68 in table III and no 39 in table II) was very unexpected The explanation is not obvious A methodological error is one possibility,

TABLE IV Relationship between renal cortical blood flow and results from split function test Summary of data from tables I and II In calculation of the mean values only patients with stenosis of the main renal artery are included

		Renal cortical blood flow				
		Lower on stenotic kidney	No difference	Higher on stenotic kidney	Mean values (ml/g min)	
Split function test	Abnormal	3 cases	4 cases	0 case	Stenotic kidney	2.5
					Non stenotic	3.2
	Normal	3 cases	6 cases	1 case	Stenotic kidney	2.8
					Non stenotic	3.0

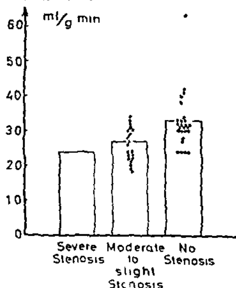


Fig 2 Renal cortical blood flow in hypertensive patients with different degrees of renal artery stenosis

differences are not statistically significant

Relationship between degree of stenosis and cortical blood flow

Figure 2 summarizes the data from I, II and III The mean value of the cortical blood flow to kidneys with normal arteries in the whole material was 3.3 ml/g min with standard deviation of 0.78 ml/g min and a standard error of the mean of 0.13 ml/g min ($n = 36$) The same figure for kidneys with a slight and moderate stenosis of the arteries was 2.7 ml/g min ($n = 29$, standard deviation 0.70 ml/g min and standard error of the mean 0.13 ml/g min) and for kidneys with severe stenosis 2.4 ml/g min ($n = 6$, standard deviation 0.92 ml/g min), standard error of the mean 0.38) The difference between blood flow in non stenotic and that in slightly to moderately stenotic arteries is significant ($t = 3.25$, $p < 0.01$) The difference between blood flow in severely and in slightly to moderately stenotic arteries is not significant ($t = 0.75$, $p > 0.1$)

Discussion

Measurement of the renal blood flow with the inert radioactive gases after injection into the renal artery was introduced by Thorburn et al (17) in dogs and by Kemp et al (7) in man The blood flow data from such measurements

9 Kjellboe H Vikgren P Oman S & Hood B Acta med scand *172* 485 1962
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17 Thorburn G D Kopald H H Herd J A Hollenberg M O'Morchoe C C C & Barger A C Circulat Res. *13* 290 1963

but an unlikely one Another possibility is the phenomenon known from animal experiments that hypertension from unilateral artery stenosis in time causes irreversible damage to the normal kidney, whereas the stenotic kidney is protected by the stenosis At least two in the present series (nos 16 and 39) had suffered from hypertension for many years, but on the other hand some of the other patients in the material also had had long standing hypertension without showing such a reversed ' flow pattern

A statistically significant relationship between the degree of stenosis and the cortical blood flow was not found

Measurement of the renal blood flow with the xenon 133 wash-out technique may be of value in the evaluation of hypertensive patients with renal artery stenosis The results of such measurements are not always consistent with the results from the split function test Thus the flow-rate measured with xenon-133 represents a new parameter in the evaluation of a renal artery stenosis Results from follow up studies after reconstructive surgery are therefore necessary before a final judgement of the clinical value of this measurement can be made

Summary

With the xenon 133 wash out technique the renal cortical blood flow was measured in 31 patients with hypertension and renal artery stenosis

In five controls the renal cortical blood flow varied from 3 6 to 6 3 ml/g min

In four of eight patients with abnormal split function test there was a significant difference between the renal cortical blood flow to the stenotic and to the non-stenotic kidney A significant difference was also present in four of ten with normal split function test This difference — four out of eight versus four out of ten — between the two groups is not statistically significant

For the whole series the renal cortical blood flow in kidneys with normal arteries was on average 3 3 ml/g min (n = 36, SE of mean 0 13) The same figure for kidneys with slight and moderate stenosis of the arteries was 2 7 ml/g min (n = 29, SE of mean 0 13) and for kidneys with severe stenosis 2 4 ml/g min (n = 6, SE of mean 0 38) The difference between renal cortical blood flow in kidneys supplied by normal arteries and that in renal tissue supplied by slightly to moderately stenosed arteries is significant ($p < 0 01$) The difference between blood flow in severely and in slightly to moderately stenotic arteries is not significant ($p > 0 1$)

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Studies on the Mechanism of Tussive Syncope

By

Asger Pedersen Erik Sandoe Eigill Hvidberg and Michael Schwartz

Tussive syncope i e fainting in association with paroxysms of coughing, is a conspicuous and well-defined syndrome, and not a very rare phenomenon More than 300 cases have been published since it was first described by Charcot in 1876 (3, 4) and clinical experience suggests that tussive syncopes and initial signs of such are even more frequent than sometimes supposed

Clinically, tussive syncope is characterised by occurring chiefly in men most often in the age class of 40 to 50 The majority suffer from chonic bronchitis and many also from pulmonary emphysema The typical patient' is a powerfully built, often overweight stenic person who is a heavy smoker and appreciates good meals and beverages The fainting fit is always associated with a paroxysm of coughing often occurring after few but vigorous coughs A very sudden onset of giddiness is followed directly by loss of consciousness Usually the patient will recover consciousness and be perfectly lucid immediately afterwards The paroxysm of coughing is not preceded by aura or other symptoms, nor attended by passage of faeces or urine, biting of the tongue or convulsions There is no post paroxysmal urge for sleep or other mental or somatic change The paroxysm often comes on so suddenly that the patient has no chance of taking precautions against a fall which may cause injuries

Many theories have been advanced concerning the mechanism of this syndrome (5) Regarding the causes, attention has been concentrated particularly on acute changes in the central nervous system or in the blood circulation, within recent years to an increasing extent on the latter The object of the present study has been to investigate the haemodynamics in patients during the fits or under conditions corresponding to those present during the fit in order thereby to elucidate the mechanism

Material

The series under review comprises five patients with clinically typical tussive syn

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copes Four of these are included in Hvidberg and Schwartz clinical report from 1962 (6)

Their histories will be briefly reported

Case 1 54 year old textile worker previously in good health During the past 15 years he has experienced increasingly frequent periods of bronchitis also as febrile attacks and progressing dyspnoea on exertion For many years the patient has been a heavy smoker During the past 6 or 7 years the paroxysms of coughing have frequently been associated with fainting fits usually following short but violent spells of coughing, though at times after prolonged coughing The state of unconsciousness during these paroxysms was presumably always of short duration, but the falls frequently caused minor injuries

Physical examination revealed no abnormalities beyond clinical and radiographic signs of pulmonary emphysema The electrocardiogram showed a QRS axis close to 90 and large S waves in the precordial deflections to V_4 The blood pressure was normal During his most recent stay in hospital he also presented rather pronounced signs and symptoms of bronchitis and, in addition a moderately raised E S R and mild leucocytosis On admission the arterial blood showed a somewhat reduced oxygen saturation (90 %) and a somewhat raised pCO_2 (57 mm Hg) but these values changed to normal in the course of three days (92 per cent and 26 mm Hg)

Case 2 A taxidriver aged 51 who is a heavy smoker and obese In his youth he had been in good health For the last 10 years he has been suffering from chronic bronchitis with progressive dyspnoea on exertion During the first years of illness particularly paroxysms of coughing had frequently been associated with giddiness and singing in the head and on several occasions also with transient fainting However during recent years the patient feels able to control these attacks and at least avoid fainting by suppressing the cough Within the past three y[illegible] tient has fainted only once in relation to a paroxysm of coughing

Physical examination during his recent stay in hospital revealed no abnormality beyond radiological signs of moderate emphysema. The patient was powerfully built and rather obese The E S R was moderately raised, but B P E C G and X ray of heart and lungs were normal The patient s driving licence had been revoked during a stay in hospital 5 years previously, at a time when he had numerous tussive syncopes

Case 3 A master joiner, aged 48 with about 20 years history of bronchitis particularly pronounced in winter He is a moderate smoker Within 48 hours he had for the first time four typical fits of syncope during paroxysms of coughing and was admitted to the Neuromedical Department Examinations in this department disclosed no neurological defects to account for the fits The patient was transferred to the Medical Department where clinical examination revealed no disorders of the heart or lungs The B P and E C G were normal and X ray showed normal conditions, except for some scattered fibrous tracts in the lungs

Case 4 Female assistant at restaurant aged 48 previously in good health Never pregnant She is a heavy smoker and very obese During the past 10 to 15 years the patient has had long periods of coughing and expectoration during winter Within the past 6 years she has in addition had three or four fits doubtlessly of tussive syncope and another fit shortly before admission Each time the fainting was associated with a particularly violent paroxysm of coughing The patient suddenly dropped and found herself lying on the floor

Ph[illegible]al examination revealed no disorders [illegible] or lungs T[illegible] E C G was normal [illegible]ed a r[illegible]d heart and nor[illegible] the B[illegible]al The E S R [illegible]ly [illegible]ddition X ray [illegible]enal ulcer

Case 5 A man aged 56 who had previously been in fairly good health He had for several years been suffering from symptoms of chronic bronchitis with frequent brief fainting fits in association with paroxysms of coughing

This patient had only been seen while in hospital 5 years before his present stay Physical examination then showed a powerfully built and obese man with no clinical signs of disorders of the lungs or heart Pulmonary function tests also showed normal conditions and the oxygen saturation and carbon dioxide tension in the arterial blood were normal The B P and Hb were normal X ray of the thorax and E C G showed no definite abnormality The patient had for some time been under treatment with an anticoagulant owing to recurrent thrombophlebitis of the legs

Methods

All the stated patients were subjected to haemodynamic investigations comprising simultaneous measurements of pressure in both the pulmonary and systemic side of the circulation In two of the patients of the present series (cases 1 and 5) such measurements had been performed 5 years before the conclusion of the case history while in the remaining cases the pressures were measured at the time of conclusion of the case histories

Pressures were measured through two or three catheters in the femoral or the external iliac artery in the right heart and pulmonary artery and in the opposite brachial vein within or outside the thorax and in one of the patients (case 4) through a cannula in the cubital vein In one (case 1) a reference intrathoracic pressure was measured at the same time through a catheter introduced into the eosophagus

The pressure in the femoral artery was always measured simultaneously with two other pressures at varying sites in the pulmonary circulation the right half of the heart and the systemic veins and in one instance the oesophagus All these pressures were measured both during a graded Valsalva manoeuvre and during a paroxysm of coughing provoked by smoking The patient was required to make the coughing as violent and protracted as possible Valsalva s manoeuvre consisted in the patient blowing a mercury or water column up to known height which he himself observed and keeping it there as long as possible using both the thoracic and the abdominal muscles The height of the pressure and the duration of the experiment were marked on the curves Three of the five patients (cases 1 2 and 5) fainted several times while the pressures were being measured either during the provoked coughing or during the Valsalva manoeuvre or during both. In two patients (cases 3 and 4) fainting could not be provoked neither by coughing nor Valsalva s manoeuvre

Results

The Valsalva manoeuvre and coughing are in principle parallel conditions Both procedures comprise a pronounced rise of the intrathoracic pressure and consequently also of the pressure on the fairly large proportion of the circulation situated within the thorax The remaining part of the circulatory bed continues to be surrounded by the atmospheric pressure, and the pressure rise here will be determined by the transmission of the intrathoracic pressure excess through the aperture of the thorax to the extrathoracic vessels

In accordance with these considerations the pressure measurements performed in our series showed a basically similar pattern of changes during coughing and the Valsalva manoeuvre

Fig 1 illustrates four simultaneous pressure measurements from one of the patients At the top is shown the pres

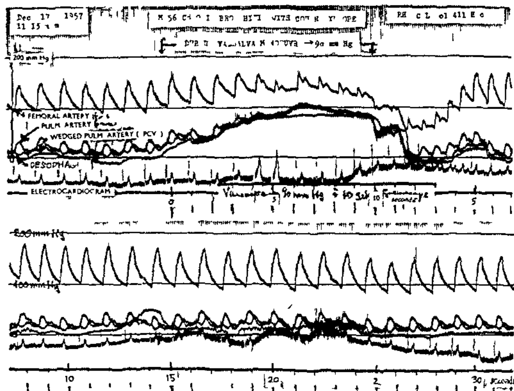
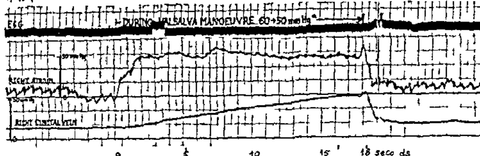


Fig 1 Multiple measurements of blood pressure in a man aged 56 with chronic bronchitis and tussive syncopes The pressures all refer to the same base and are from above to below the femoral artery the pulmonary artery the PCV pressure and the pressure in the oesophagus as a measure of the intrathoracic pressure At the bottom an electrocardiogram All the pressures were measured during Valsalva s manoeuvre No fainting As for discussion see text

sure in the femoral artery and below two intrathoracic pressures, viz wedge and pulmonary artery pressures, and the pressure in the oesophagus, all measured during the Valsalva manoeuvre The patient blew the fluid column up to a pressure of about 90 mm Hg for 10 sec He felt some giddiness during the procedure, but there was no fainting

It is plain to see that the wedge pressure and the pulmonary artery pressure rose parallel to the intrathoracic pressure, measured by that in the oesophagus It is also noticed that the pulse pressure in the pulmonary artery fell rapidly so as to become almost abolished probably indicating a decreasing stroke volume of the right ventricle towards zero Immediately after the conclusion of the Valsalva manoeuvre the pressures fell to their usual levels The pressure in the systemic artery was likewise greatly influenced, as seen in the upper curve During the first part of Valsalva s manoeuvre the mean systolic arterial pressure displayed a rise in the intrathoracic pressure Soon afterwards the pulse pressure decreased considerably, suggesting that the stroke volume of the left ventricle also was declining towards zero Immediately after the conclusion of the Valsalva manoeuvre the arterial pressure



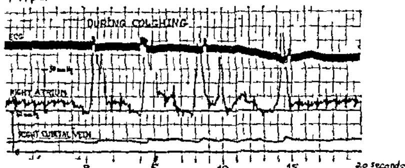


Fig 2 Simultaneous measurements of pressures in the right atrium and in a brachial vein during Valsalva's manoeuvre and during coughing Note that these curves unlike those of the other two figures have no common zero level No fainting Cf text

fell to almost half of its normal value and then both the pulsations and the pressure level began to rise For about 30 seconds the systolic as well as the pulse pressure were markedly raised presumably indicating a transitory increase of the stroke volume of the left ventricle until the distribution of the blood volume had been rearranged and probably a reflectory increase in the peripheral resistance also The electrocardiogram (lower curve) shows that the heart action was undisturbed beyond a transitory tachycardia after the conclusion of Valsalva's manoeuvre and a few extrasystoles during the manoeuvre Bradycardia or asystole were never recorded

In fig 2 the results are seen of simultaneous measurements in the right atrium and a peripheral vein in another patient partly during Valsalva's manoeuvre and partly in relation to coughing Valsalva's manoeuvre brought about an instantaneous and plateau like rise of the pressure in the right atrium closely following the intrathoracic pressure The pressure in the peripheral vein on the other hand rose only gradually, having taken 16—17 seconds to reach the same level as that in the intrathoracic veins During this period the circulation

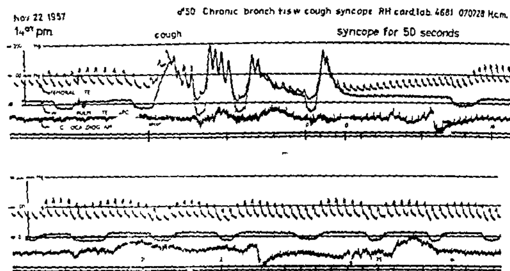


Fig 3 Simultaneous [illegible] of pressures in a peripheral pulmonary artery and a wedged pulmonary artery PCV [illegible] ell as an electrocardiogram during a paroxysm of coughing followed by fainting Cf text

must have been [illegible] letely interrupted at the opening [illegible] thorax and the venous flow [illegible] art have ceased entirely Th[illegible] ourse, lead to a decreasing [illegible] ume first of the right and the [illegible] the left ventricle, and a fall of [illegible] rial pressure, as shown in fig [illegible] ing was seen to cause an even hig[illegible] e of the pressure in the right atrium while as shown in the lower part of fig 2 the peripheral venous pressure did [illegible] have time to rise correspondingly During coughing the circulation must there[illegible] re also have been totally interrupted at [illegible] he thoracic opening

Fig 3 illustrates the results of similar measurements of the pressures in a peripheral artery and the pulmonary artery during a violent paroxysm of coughing with associated tussive syncope and transitory complete loss of consciousness These measurements disclosed principally the same changes, only to a very pronounced degree The pressure in the intrathoracic vessels rose to 150—200 mm Hg during the coughs For some time the rise kept pace with that of the pressure in the peripheral arteries, suggesting that the stroke volume of the left ventricle had fallen towards zero During the inspirations between the coughs the intrathoracic pressure fell to well under zero and the arterial pressure to nearly zero The patient fainted after no more than about 10 seconds of coughing During the next 10 seconds the arterial and the pulse pressure remained at a very low level while the pressure of the pulmonary veins (measured as the "PCV pressure") was at a somewhat higher level than normally After another 10—15 seconds the pressures returned to normal and the

patient recovered consciousness However it took him nearly one minute to become fully conscious

Discussion

As stated above, many attempts have been made to explain the mechanism of the characteristic syndrome called tussive syncope, but few of these have received experimental support

One of the earliest and most enduring explanations was that of a reflex mechanism released from the larynx (ictus laryngis) This is thought to be analogous with the fainting fits that may in very rare cases be caused by pressure against or morbid processes in the larynx the pharynx or the carotid sinus in which cases a pronounced bradycardia or asystole is released through the vagus nerve Such disturbances in cardiac rhythm have never been demonstrated, however, during a tussive syncope, and the results of the investigations reported above militate against this hypothesis In all the cases described here and in other papers a normal heart action was always recorded in the electrocardiogram during the attack itself

The last stage of the syncope must in all cases be one of a transient, severe hypoxia in the brain owing to a failing blood supply A few writers (9) suppose that a raised pressure in the brain itself during coughing leads to hypoxia owing to the impossibility of expansion in the closed cranial cavity This is however, a purely speculative hypothesis Kerr and Eich (7) incline to the view that the circulatory changes in pressure release the syncope *via* a pronounced and rapid rise in the pressure of the cerebrospinal fluid, which they believe to cause a kind of internal concussion of the brain One of the most important arguments in support of this view is the fact that the fits of syncope come on sooner (within 9 seconds (cf 7)) than might be expected if caused by the haemodynamic changes In our experience however, the circulatory changes occur in fact so quickly that a time factor is unlikely to favour the theory of concussion of the brain more than that of a direct effect on the cerebral blood flow of serious changes in the central circulation (fig 3) McCann et al (8), on the basis of measurements of the intrapulmonary pressure advanced the hypothesis that compression of pulmonary capillaries during the cough, together with a peripheral vasodilatation and collapse of the large thoracic veins, might be the cause of the cerebral anoxia in tussive syncope However, the present investigations plainly showed (fig 1) that the rise in pressure affects all the pulmonary vessels equally and all the extravascular pressures round all the vessels within the thorax The important difference in pressure arises between the intra- and the extrathoracic veins where the circulation must at any rate have been arrested That the peripheral vasodilatation is unlikely to play any part in the release of syncope is evident from some investigations by Kerr and Eich who could provoke syncope even in patients wearing an anti G suit (7)

The results of our study closely agree with those of various previous investigations into haemodynamic values in and outside the thorax during both Valsalva's manoeuvre and coughing (1 2 10) All

these workers demonstrated gross changes in the central circulation under these circumstances, changes which may be briefly described as follows Throughout the intrathoracic portion of the circulation the pressure rises abruptly and excessively exactly parallel with the intrathoracic pressure In the peripheral veins the rise in pressure proceeds at a slower rate, presumably because the venous system with its larger volume and more elastic walls requires a very considerable increase of the blood volume before the pressure rises It takes some time for such large amounts of blood to be transferred to the venous system Consequently, the peripheral venous pressure will for about 10 seconds or more lie at a lower level than the pressure in the intrathoracic veins This means that the circulation must have been completely arrested at the opening of the thorax, i e the venous blood flow to the heart has ceased Accordingly the stroke volumes of the ventricles decrease rapidly (2), first the right and then the left, resulting in a marked fall of the systemic arterial pressure Simultaneously the pressure in the systemic veins is slowly increasing and the pressure gradient from arteries to veins in the systemic circulation decreases resulting in an impairment of the peripheral blood flow These changes in the central and the peripheral circulation are so pronounced — as seen in figs 1–3 — that they must be considered to explain why the blood flow through the brain may fall below a certain critical level for each patient On the other hand, none of the haemodynamic investigations gave results favouring the theories of a vagus nerve reflex action on the heart or of compression of intrathoracic portions of the circulation Further, as stated above a direct effect of the high pressure on the central nervous system need not be considered as the cause of the fainting The changes in the circulation develop so rapidly that they may very well account for the clinically observed prompt loss of consciousness It must be stressed that the very serious circulatory changes described are normal reactions to Valsalva's manoeuvre and coughing It therefore remains to be explained why they lead to loss of consciousness in some patients and not in others Individual differences in a presumed cerebral threshold value may, perhaps, play a part However both clinical observations and the measurements of pressure (2, 10) suggest that among the many variable factors to be considered is the patient's constitution within a habit of coughing very vigorously Pulmonary emphysema and heavy smoking are frequent findings It is therefore most likely the degree of the circulatory change which characterises such patients and causes them in particular to have yet another symptom added to their troubles, namely that of fainting during paroxysms of coughing

Summary

Five patients with typical tussive syncope were subjected to haemodynamic investigations by simultaneous measurements of three or four pressures at different sites of the circulation partly during paroxysms of coughing and partly during a graded Valsalva s manoeuvre The results support the view that the

loss of consciousness during the paroxysms of coughing can be explained solely by the circulatory changes On the other hand they militate against theories of reflex actions, compression of the pulmonary capillaries, and a direct effect of sudden changes in pressure within the central nervous system

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Coronary Heart Disease in a Population in the South of Sweden

By

G Biorck, G Blomqvist, P Hall, B W Johansson, Eva Larsson, H Malmros J Sievers and B Swahn

In 1954, Keys, White, Malmros, Biörck, Swahn et al undertook a comparative study of the incidence of coronary artery disease within certain population groups in Naples Italy, and Malmo, Sweden The Naples sample was composed of steel workers firemen, and members of the Rotary Club The Malmo sample was made up of ship yard clerks and workers and firemen mainly from the Malmö Fire Department Partial results have been published (3, 4 10) but no report of the entire Swedish material has yet been made A 6 year follow up of the Malmo group was done in 1960 The group is comparatively small, and it is not expected to yield any dependable *frequencies or* correlations until it has been followed for at least 10 years The present study was done primarily to record any changes in the group during the follow up period in regard to characteristics previously known to be correlated with coronary artery disease

Submitted for publication October 7, 1965

Material and methods

In 1954 invitations to participate in a heart study were sent out to certain labourers and clerks at Kockum s ship yard in Malmö as well as to all firemen in Malmo Halsingborg and Lund The invitations mentioned the need for a detailed investigation of the frequency of coronary disease in a Swedish population and the men were requested to volunteer for such a study 464 persons accepted 162 clerks and 152 labourers at the Kockum ship yard in Malmo and 150 firemen in the three southern Swedish towns As far as the method of selection is concerned the sample cannot be said to be representative for the ship yard employees, but is so for the firemen

The age and occupation frequency distributions are given in table I

The 464 volunteers reported in 1954 to the Malmö General Hospital Cardiac Clinic where a thorough physical examination was given All subjects were fasting A 12 lead resting ECG was taken and a blood sample withdrawn for cholesterol and alpha and beta serum lipoprotein analyses The same subjects except 8 who had died and 7 who either declined participation or could not be reached by invitations that is

TABLE I Sample composition Age distribution at the 1954 examination

Age 1954	Kockum shipyard employees	Firemen	Total
30	56	38	94
30–39	85	62	147
40–49	87	37	124
50–59	82	13	95
60–69	3	–	3
70–>	1	–	1
Total	314	150	464

449 persons were reexamined in 1960 (follow up = 98 7 per cent) All subjects in the group of 7 men who were not re examined were alive at the time of the follow up study We have no information on their present state of health

The 1960 re examinations were carried out in the same way as in 1954 except for ECG s being done not only at rest but also during exercise for 95 8 per cent of the 457 subjects examined A simple step test (a 30 cm high "step 20 ascents descents per minute, for 3 minutes) was used for the exercise ECG A 12 lead standard ECG was recorded both during rest and immediately after exercise and analyzed according to the Minnesota Code (6), modified 1964 (13) with regard to ST depressions (A new item IV 0 was introduced to denote a horizontal or downward sloping ST depression of 1 mm or more) All ECG s were read and coded by the same person Eight subjects who were not able to appear in Malmo were studied at other hospitals through the courtesy of colleagues The cholesterol determination method used at the Lund Laboratory in 1960 differed from that used in 1954 (Anderson and Keys modification (2) of the method given by Abell et al (1)) A series of duplicate determinations done on 30 samples by both the 1954 and the 1960 methods showed a linear relationship between the results All values have been recalculated according to the 1954 method standards

Results

The frequency of new cases of clinical coronary disease

Of the 8 deaths during the period 1954—1960, 5 were the result of malignant diseases One man died of coronary artery disease There was also one suicide and one accidental death (drowning) All information on the causes of death is based on death certificates

Of the total sample of 449 re examined subjects, 4 exhibited clinical symptoms and electrocardiographic changes indicating coronary artery disease in 1954 Thus there remained 445 men free from coronary artery disease in 1954 who were re examined in 1960

Nine of these 445 exhibited evidence of clinical coronary artery disease occurring between 1954 and 1960 Two had suffered infarcts, documented by hospital records Three additional men had been admitted to a hospital with probable but not positively verified infarcts The remaining 4 subjects produced a typical history of angina pectoris, and their 1960 resting ECG's showed changes compatible with coronary disease

Twelve cases were classified as probable cases of coronary artery disease The diagnoses in these 12 men rest entirely on the medical history All experienced chest pain of anginal type upon exertion

TABLE II Occurrence of coronary disease and deaths in all age groups in the follow up material

Age 1960	Healthy 1954 and 1960	Developed coronary disease during 1954—1960		Coronary disease 1954	Death during 1954 1960	Total
		Probable	Definite			
20—29	12	—	—	—	—	12
30—39	136	1	—	—	1	138
40—49	117	2	2	—	—	121
50—59	103	6	3	2	5	119
60—69	55	3	4	2	2	66
70—>	1	—	—	—	—	1
	424	12	9	4	8	457

The remaining 424 men presented no history of coronary disease at the 1960 examination

In this material, no patient was classified as either probable or positive coronary disease on the basis of ECG changes alone

Age distributions within the 3 groups are given in table II

Blood pressure

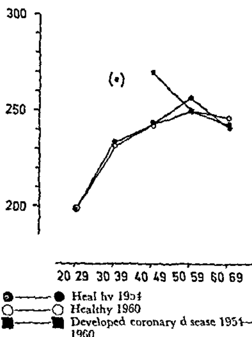
19 4 per cent of the healthy men versus 25 per cent of those with *probable coronary disease* and 33 per cent of those with *definite coronary disease* exhibited a diastolic pressure of 100 mg Hg or more Thus the tendency toward higher values in the coronary disease group is obvious although the differences themselves are not statistically significant

Blood lipids

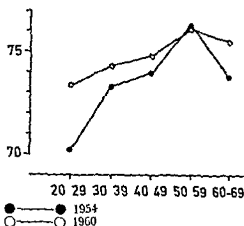
The serum cholesterol values given in fig 1 show the means for healthy men by age group in 1954 to be practically identical with those for 1960 Patients in the 30—39 and 40—49 age groups who developed coronary disease during the six years had higher cholesterol values but there were no differences in the older age groups However the number of coronary cases under 50 years of age was only 6 No significant differences were obtained between the coronary cases and the healthy subjects for alpha and beta lipoproteins

Anthropometric data

Fig 2 shows body weight at both examinations to have been highest for the 50 59 year old group No great change has occurred during the follow-up period for the group as a whole but the values obtained in 1960 were consistently somewhat higher and the age differences decreased slightly No difference in 1954 weights could be shown between those subjects developing coronary disease during the 6 year period and the remaining healthy subjects Those who later developed *definite coronary disease* had a mean weight in 1954 of 74 8 kg The corresponding figure for the *probable coronary disease*



F g 1 Serum cholesterol accord ng to age group



F g 2 Body we ght accord ng to age group Men w th no s gn of coronary disease

group was 73 6 kg and the age corrected mean for the *healthy male subjects* was 74 8 and 75 0 kg respectively Also the individual weight variation throughout the 6 year period showed no particular difference between the healthy and coronary disease subjects Most men increased somewhat in weight corresponding to the rise in weight with increased age up to 50—59 years of age as given in fig 2 but there were large

TABLE III Ind v dual we ght variat ons n all age groups Increase or decrease in kg

	Age 1954	−10	−5 9	−3 4	1 2	±0	+1 2	+3 4	+5 9	+10
Heal hy	20 29	–	1		–	3	2	2	2	2
	30 39		6	6	11	9	30	28	26	11
	40 49	–	5	11	14	14	37	16	14	4
	50 59	1	4	9	12	26	23	15	13	–
	60 69	1	4	12	7	6	11	4	7	1
	70 79	1								
Total		3	20	38	44	58	103	65	62	18
Defin te or	30 39					–		1		–
Probable	40 49		1		–	–	1	–	1	–
Coronary	50 59		–	1	1	1	4		1	–
Disease	60 69		1	1	3	1		1	1	–
Total			2	2	4	2	5	2	3	–

TABLE IV ECG changes according to the modified Minnesota code

Age	20–29	30–39	40–49	50–59	60–69	70
A ECG 1954						
Number	*86*	*140*	*121*	*95*	*6*	
Q and QS						
group 1			1			
group 2		1	2	1		
group 3	1	2	2			
Left axis deviation		1	1	5		
High R V_{5-6}	10	6	3	5	1	
ST depressions						
group 0		1				
group 1		1				
group 2			1	1		
group 3				3		
T changes						
group 1						
group 2						
group 3		2	1	6		
Prolonged S-T interval		1	1			
Complete bundle branch block						
left						
right			1			
Premature beats	1					
Sinus bradycardia	7	3	7	3		
Sinus tachycardia		1				
B ECG 1960						
Number						
rest	12	136	120	114	62	6
exercise	12	136	120	110	54	5
Q and QS						
group 1			2	1	1	
group 2		1	1	3	1	
group 3		3		1		
Left axis deviation		2		6	6	1
High R V_{5-6}		6	6	3	2	
ST depressions						
group 0		1	1		2	
group 1			1	2		
group 2			1	8	3	
group 3				3	1	1

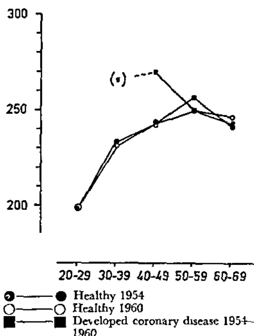


Fig 1 Serum cholesterol according to age group

group was 73 6 kg, and the age corrected mean for the *healthy male subjects*

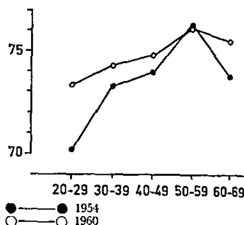


Fig 2 Body weight according to age group Men with no sign of coronary disease

was 74 8 and 75 0 kg, respectively Also, the individual weight variation throughout the 6 year period showed no particular difference between the healthy and coronary disease subjects Most men increased somewhat in weight, corresponding to the rise in weight with increased age up to 50—59 years of age, as given in fig 2, but there were large

TABLE III Individual weight variations in all age groups Increase or decrease in kg

	Age 1954	−10	−5–9	−3–4	−1–2	±0	+1–2	+3–4	+5–9	+10
Healthy	20−29	–	1	–	–	3	2	2	2	2
	30−39	–	6	6	11	9	30	28	26	11
	40−49	–	5	11	14	14	37	16	14	4
	50−59	1	4	9	12	26	23	15	13	–
	60−69	1	4	12	7	6	11	4	7	1
	70−79	1	–	–	–	–	–	–	–	–
Total		3	20	38	44	58	103	65	62	18
Definite or	30−39	–	–	–	–	–	–	1	–	–
Probable	40−49	–	1	–	–	–	1	–	1	–
Coronary	50−59	–	–	1	1	1	4	–	1	–
Disease	60−69	–	1	1	3	1	–	1	1	–
Total		–	2	2	4	2	5	2	3	–

cardiogram at rest was of no value for the prediction of future coronary heart disease

Acknowledgements

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Multiple Pulmonary Artery Aneurysms

By

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Pulmonary artery aneurysms located peripherally in the lungs are rare In the literature only 34 cases are recorded, most of which have been single Only a few have been investigated with pulmonary angiography On conventional roentgen films single or multiple pulmonary artery aneurysms located peripherally in the lungs have the same appearances as other vascular anomalies and occasionally give rise to difficulties in distinguishing them from other soft tissue masses This is especially the case when the pulmonary aneurysms are acquired and arise during a limited period

Case report

The patient a 40 year old carpenter had enjoyed good health when in 1952 a routine X ray film of the chest during military service revealed pulmonary tuberculosis In 1956 progression of the pulmonary lesions was observed requiring 4 mon hs hospitalization in 1957 Though cultures for tubercle bacilli were repeatedly negative he was treated with PAS streptomycin and Isoniazid

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In 1958 he had a small haemoptysis which was thought to be related to the tuberculous disease and he complained of dyspnoea on exertion Digitalis treatment was started

In 1962 he was admitted to the Medical Department because of increasing dyspnoea on walking Physical examination showed moderate cyanosis of the lips and fingernails but no clubbing There was a slight dyspnoea in the recumbent position The cardiac apex beat was widened and lifting and extended into the anterior axillary line Auscultation revealed a narrowly split second heart sound a faint systolic murmur over the apical region and frequent premature beats The cervical veins were not distended and the liver was not enlarged The peripheral pulses were normal The blood pressure was 115/85 mm Hg Physical examination of the lungs was normal as well as ophthalmological and oto-rhinological examinations

Laboratory findings

The erythrocyte sedimentation rate was 34 mm/hour The white cell and the differential counts were within normal limits with 2—3 % of eosinophils There was no anaemia

Serum cholesterol was 180 mg per 100 ml Serum sodium potassium chlorides and calcium concentrations were normal

TABLE I Heart catheterization data at rest and during work

	A VO_2 diff (ml/l)	O_2 up take (ml/min)	Card output (l/min)	Heart rate	Stroke volume (ml)	Art O_2 sat (%)
At rest	42	313	7 4	78	92	96 7
Work 200 kpm/min	65	688	10 6	111	95	94 5
Work 400 kpm/min	88	1,025	11 6	114	80	93 4

Explanation of symbols

V_E = Resp minute volume
R V = Right ventricle
P A = Pulm artery
PCV = Pulm capillary venous pressure

Urine analysis was normal and clearance studies revealed normal glomerular and tubular function Serological examinations including Wassermann s reaction antistreptolysin and antistaphylolysin titres as well as the acryl test for rheumatoid factor were normal LE-cells could not be demonstrated by *repeated examinations*

Search for acid fast bacilli in sputum smears was repeatedly negative as well as cultures for tubercle bacilli from gastric lavage

A muscle biopsy from the right brachioradialis muscle showed no pathological changes of the blood vessels

Cardio pulmonary function investigation

The ECG demonstrated a left bundle branch block and frequent ventricular premature beats which disappeared during work test performed on a bicycle ergometer and reappeared in connection with supraventricular premature beats after exercise The physical working capacity (calculated as the working intensity at a heart rate of 170 beats/min PWC_{170} (14) was 600/kpm/min which was low in relation to both the total amount of haemoglobin and the radiologically determined heart volume (8) While the total amount of haemoglobin was normal in relation to body weight the heart volume was large in relation to the total amount of haemoglobin thus demonstrating cardiac enlargement Pulmonary function investigations revealed disturbances of the obstructive pattern of a mild degree Right heart catheterization and direct arterial pressure recording performed in the supine position at rest and during work revealed the data summarized in table I

The most striking findings were a high pulmonary artery wedge pressure (the value at rest being the mean of 5 measurements in different parts of the lung range 21—28 mm Hg) pulmonary hypertension during exercise and a small stroke volume in relation to heart volume (the stroke volume predicted (4 6) from heart volume was 126 ml) The arterial oxygen saturation was mainly normal at rest and during exercise

Roentgenological examinations

The first pulmonary examination available was performed in 1952 In the upper lobes of both lungs tuberculous changes without cavitation were evident There were no changes of the pulmonary arteries or their branches The heart was not enlarged (fig 1) In 1956 pathological changes were noted

		Pressure									
		(cm H_2O)	(mm Hg)								
			R V		P A				B A		
$\dot{V}_E$ (l/min)	Resp rate	Intra oesoph	S	D	S	D	M	PCV	S	D	M
14	15	−7/±0	30	10	30	12	17	22	108	73	85
22.5	19	−10.5/+1	45	10	46	25	32				87
35	26	−18/+1	47	8	47	26	34	27			86

B A = Brachial artery
S = Systolic
D = Diastolic
M = Mean

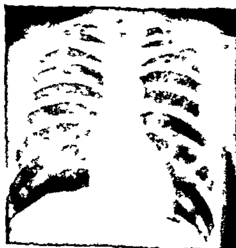


Fig 1 Chest X ray 1952 Tubercuious mainly fibrotic changes in the upper lobe of both lungs Pulmonary arteries and heart without pathological changes



Fig 2 Chest X ray 1962 The appearance of spindle shaped soft tissue masses in both lungs Enlargement of the heart

peripherally in the lungs which were though to be due to a progression of the pulmonary tuberculosis The changes appeared as spindle shaped sharply-defined soft masses, mainly in the right lung A dilatation of the main branches of the pulmonary arteries was also evident In 1962 the heart was found to be enlarged and the peripheral pathological changes in the lungs to be increased consisting of several *spindle-shaped masses of* vascular appearance (fig 2) On tomographical examination their connection with the pulmonary arteries was evident They appeared as aneurysmal widenings of several of the peripheral branches of the pulmonary arteries on both lungs (fig 3)



Fig 3 Tomography Aneurysmal widenings of peripheral vascular branches best visible in the right lung

Pulmonary angiography was performed in connection with heart catheterization with the tip of the catheter placed in the main pulmonary artery The peripheral branches of the pulmonary artery on both sides were widened although a few of them were of normal calibre On both sides localized aneurysmal widenings of the peripheral branches were seen corresponding to the masses demonstrated on plain films and tomograms Some peripheral arteries were of a peculiar appearance with an irregular lining No local stenoses were seen in the arteries The pulmonary veins were unchanged (fig 4)

Discussion

Incidence and clinical features

Pulmonary aneurysms of the main pulmonary artery or of the two main branches are not very unusual Deterling and Clagett collected 147 cases from the literature in 1947 (3), and according to Ismein et al (5) a total of 158 cases was reported up to 1962

Aneurysms located peripherally and affecting the minor branches of the pulmonary artery, however, are rare In 1961 Charlton and du Plessis (2) found only 30 cases in the literature and added one case of their own Kopp and Green reported one case in 1962 (7) and Calenoff two cases in 1964 (1), the latter two being the only cases examined by angiography Thus, a total of 34 cases has been published up to 1964 Only a few of them had multiple or bilateral aneurysms



A B

Fig 4 A Pulmonary artery angiography B Multiple peripherally located aneurysmal widenings of the pulmonary arteries in both lungs

The aetiology of these 34 cases was considered to be mycotic or probably mycotic in 23 congenital in 8, and syphilitic and traumatic in one case each In one case it was due to embolism of suture material from a repaired patent ductus arteriosus

Thus most commonly peripheral pulmonary aneurysms appear to be of mycotic origin They may be caused by septic embolism into the pulmonary artery or its vasa vasorum, i e the bronchial arteries or by direct invasion from a suppurative focus in the lungs In younger individuals the most common cause is said to be bacterial endocarditis (11) The clinical symptoms of a mycotic aneurysm usually consist of more or less prolonged or intermittent periods of fever cough, haemoptysis, chest pains and dyspnoea A massive haemoptysis has often been the cause of death

Haemodynamic considerations

Three of the cases reported — two with probably congenital and one with probably mycotic aneurysms — underwent right heart catheterization All had marked pulmonary hypertension, the cause of which could not be elucidated.

In the present case the pulmonary artery pressure at rest was mainly normal but the pulmonary artery wedge pressure (PCV pressure) at rest was significantly higher than the mean pulmonary artery pressure A possible explanation may be increased flow through bronchopulmonary arterial anastomoses which may give rise to local pressure increase when a small pulmonary artery is occluded by the catheter Such bronchopulmonary anastomoses are known to occur often in scarred areas of the lung (10) and reversal of blood flow in small pulmonary arteries due to such bronchopulmonary arterial anastomoses has been demonstrated in bronchiectasis (9)

During exercise a significant rise in the mean pulmonary artery pressure occurred which could have been due to an increase of the pulmonary vascular resistance or to an increase of the left ventricular filling pressure Mitral valve obstruction as a cause of high left ventricular filling pressure could be excluded Pulmonary vascular resistance could not be assessed with certainty as the high PCV pressures cannot be considered as representative of left ventricular filling pressure However, the findings of left bundle branch block and of a reduced stroke volume in relation to heart volume as well as the absence of the normal pressure rise in the brachial artery seem to speak in favour of a rise of the left ventricular end diastolic pressure i e a relative insufficiency of the left ventricle as the main cause of pulmonary hypertension during exercise Unfortunately a definitive assessment was not possible as left ventricular catheterization could not be performed a second examination being refused by the patient

Aetiological considerations

In the absence of prolonged periods of fever, other signs of septicaemia or an embolic focus (thrombophlebitis, of congenital heart disease syphilitic infection and trauma all the aetiological possibilities mentioned in the literature can probably be excluded Moreover

there were no signs indicative of rheumatic disease or disease of the arterial system such as endarteritis obliterans and polyarteritis nodosa Small acquired aneurysms located either on peripheral branches of the pulmonary artery or on bronchial arteries passing in the wall of a tuberculous cavity are described by Rasmussen as a cause of haemoptysis in connection with advanced tuberculous changes in the lungs (12, 13) These aneurysms, however, do not belong to the group of pulmonary aneurysms under discussion as they do not acquire a size enough to be recognized on roentgen examination Moreover no cavities were found in this patient, the tuberculous changes were located in the upper lobes with the aneurysms mainly in the middle and lower portions of the lungs

While this patient presents a clear cut morphological picture the aetiology of these changes thus remains uncertain

Summary

The case of a 40 year old man is reported who developed multiple peripheral pulmonary artery aneurysms during observation for a fibrotic pulmonary tuberculosis The diagnosis was established by pulmonary angiography in connection with right heart catheterization The aetiology of the vascular changes could not be elucidated

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Side Effects of Treatment of Hypogammaglobulinaemia with Gammaglobulin

By

C Kamme, C Coster, E Hagelquist, N Kalén, H Lindholm and R Grubb

It has long been known that intravenous injection of gammaglobulin preparations is not free from risk Serious complications are known to have occurred after intravenous injection of large doses in patients with cancer (4, 18) and after intravenous injection in patients with hypogammaglobulinaemia (16, 20)

These sequelae have been tentatively attributed to allergy to the preservative (usually sodium thiomersalyl) or to some contamination of the preparation e g with silk during the manufacturing process (7) or to a so called colloidal reaction resembling that following intravenous injection of colloidal iron (19) or fat emulsions (4, 18, 27)

Judging from recent experimental investigations (2, 3), however, the side effects of treatment with gammaglobulin, which occur mainly in patients with hypogammaglobulinaemia, may be ascribable to aggregation of the gamma globulin molecules and an anticomplementary effect of the preparation

This paper is concerned with complications following the administration of gammaglobulin in the ordinary course of treatment of adults with hypogammaglobulinaemia

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Material and methods

Gammaglobulin Kabi contains 12 % gammaglobulin dissolved in 0 3 molar glycine The preparation contains minute amounts of sodium bicarbonate and traces of sodium acetate and sodium chloride It also contains a preservative sodium thiomersalyl diluted 1 10 000 The manufacturing process does not include filtration through silk

Serological tests for antibodies to gamma globulin were performed by 1 precipitation techniques (ring test and by diffusion in gel methods *ad modum* Ouchterlony) Gammaglobulin Kabi served as the antigen 2 agglutination tests using Rh positive red cells coated with at least 5 high titred in

complete anti Rh antibodies, including the anti Rh Ripley of Waller and Vaughan (27)

Case reports

Of the five cases reported below and collected from various parts of Sweden cases 1 and 2 have been described previously by Larsson et al (13) and case 3 by Lindholm (14) The side effects are briefly mentioned in those publications In the present article these case reports have been brought up to date

All five patients were adults and suffered from acquired hypogammaglobulinaemia Complications in the form of recurrent respiratory tract infections sepsis or purulent meningitis appeared between the ages of 20 and 30 years in four of them In case 3 the resistence to infections had been low since the age of 10 years

All of the patients had been treated with gammaglobulin Kabi All the injections had been given intramuscularly

Case 1 B R (This case has been described previously by Larsson et al (13)) A man born in 1922 He felt well until about 20 years of age when he began to have recurrent respiratory tract and intestinal infections Pernicious anaemia was diagnosed in 1956 and hypogammaglobulinaemia in 1957 Serum electrophoresis in 1957 showed total 6 2 albumin 4 2 α_1 globulin 0 4 α globulin 0 8 β globulin 0 7 and gammaglobulin 0 1 g/100 ml Bloodgroup O Rh No demonstrable iso agglutinins

Treatment with gammaglobulin was started in 1957 with 32 ml every 6th to 8th week The dose was divided into 4 equal fractions which were given at two hour intervals This treatment kept him practically free from infections The injections were not followed by any side effects until November 1959 when a few minutes after he had received the second fraction of the dose he reacted with cough nausea flushing stiffness of the face and neck lumbar pain and cyanosis as well as general respiratory difficulties He improved progressively after ten to fifteen minutes One hour later he had chills and the body temperature rose to 40° C The following day he was tired but otherwise afebrile and symptom free

Intracutaneous tests were performed with merthiolate as well as with gammaglobulin with and without merthiolate After twenty minutes a papule developed with surrounding diffuse redness around the site of injection of gammaglobulin and that of gammaglobulin with merthiolate A similar though weaker reaction appeared at the site of the test with merthiolate alone After 18 hours there was no longer any visible reaction to gammaglobulin alone but a reddening of the skin and a slight increase in consistency in an area of 35 $\times$ 35 mm around the site of injection of gammaglobulin with merthiolate Merthiolate alone produced reddening of the skin in a small area only (7 $\times$ 7 mm)

The results of the tests were regarded as positive for merthiolate In an attempt to desensitize the patient, increasing doses of gammaglobulin *with* merthiolate were injected subcutaneously He afterwards received 5 ml a week without complications until the autumn of 1961 when he again reacted in the above mentioned way though less severely

Treatment was continued as before with a dose of 5 ml a week but in an attempt to counteract any side effects the patient was now given a tablet of Benadryl one hour before the injection

In May 1962 the dose was reduced to 4 ml every 14th day and proved sufficient to keep the patient free from infections No complications occurred until the summer of 1963 when lumbar pain chills and fever occurred a few minutes after an injection In March 1964 the patient reacted during the actual injection (amount injected about 1 5 ml) this time with flush cough and severe lumbar pain radiating into the legs A quarter of an hour later he had a rigor The following day he felt tired but was otherwise symptom free

No antibodies to gammaglobulin could be demonstrated in the patient s serum examined one month later

Because of this severe reaction he has refused gammaglobulin since March 1964 During the last year he has been largely free from infections

Case 2 I F (This case has been described previously by Larsson et al (13)) A woman born in 1919 with known pernicious anaemia since 1944 She had repeatedly had respiratory tract infections with pneumonia otitis and sinusitis Since 1955 she had increasing respiratory difficulties with dyspnoea and cyanosis Roentgen examination has shown bronchiectasis Hypogammaglobulinaemia was diagnosed in 1955

Serum electrophoresis in 1957 showed the following values Total 4 9 albumin 3 2 α_1 globulin 0 4 α_2 globulin 0 6 β globulin 0 5 gammaglobulin 0 2 g/100 ml Blood group O Rh+ No demonstrable isoagglutinins In February 1957 treatment was started with 16 ml of gammaglobulin every 14th day This treatment was not followed by any complications until May 1958 when she had severe dyspnoea for a few minutes immediately after the end of the injection During the following 2 years during which treatment was continued no complications occurred In January 1960 severe dyspnoea cyanosis and severe anxiety occurred one minute after the end of an injection The symptoms lasted for about 10 minutes

In February 1960 intracutaneous injection of 0 1 ml of the same preparation that had caused the side effects produced no reaction whether given alone or with merthiolate Five days later subcutaneous injection of 0 2 ml of the gammaglobulin alone (of the same preparation) produced distinct redden ing after 12 hours When injected subcutaneously an equal amount of merthiolate alone produced no reaction Subcutaneous tests with gammaglobulin alone were repeated after one week and three weeks and gave the same positive result At the time of the last test she had been receiving treatment with cortisone for 5 days One month later intracutaneous subcutaneous and intramuscular tests with another preparation without merthiolate proved negative Treatment was then continued with gammaglobulin without merthiolate in the same dose as before and no complications attributable to the treatment appeared until the patient s death from respiratory insufficiency in June 1963

Case 3 G A (This case has been described previously by Lindholm (14)) A man born in 1935 was essentially healthy until 1944 He had been vaccinated against small pox without complications at the age of 6 years In 1944—1956 he had repeated respiratory tract infections He was admitted to hospital in 1948 for investigation of petechiae on the trunk and arms Examination on admission revealed splenomegaly anaemia thrombocytopenia and leukopenia Blood group O Rh— No demonstrable isoagglutinins The patient was subjected to splenectomy after which the blood picture became normal Purulent meningitis in 1948 and 1958 Serum electrophoresis was not done until 1958 and then showed the following values total 5 9 albumin 3 8 α_1 globulin 0 4 α-globulin 0 9 β globulin 0 7 and gammaglobulin less than 0 1 g/100 ml

Treatment with a total monthly dose of 24 ml gammaglobulin given as three equal fractions within one to two hours was started in July 1958 and was not followed by any complications until December that year when 5 minutes after the first dose of 8 ml he reacted with nausea vomiting and cold sweats After a further half hour he had a rigor and the temperature rose to 38 5 C No fall in blood pressure Some hours later he felt better An intracutaneous test one month later with gammaglobulin with merthiolate diluted 1 10 in physiological saline produced bo reaction No antibodies to gammaglobulin could be demonstrated in the patient s serum December 1958) Treatment was continued with gammaglobulin with merthiolate in the same dose as before and no complications occurred until June 1961 when he reacted with the same symptoms as in 1958 about 20 minutes after the third injection The next injection 1 month later was preceded by a test dose of 2 5 ml

TABLE I Symptoms of untoward reactions

	Case 1	Case 2	Case 3	Case 4	Case 5
Flush	+			+	
Feeling of tension in the chest				+	+
Lumbar pain	+			+	+
Dyspnoea	+	+			
Cough	+				+
Cyanosis	+	+		+	
Nausea and vomiting	+		+	+	
Diarrhoea			+	+	
Loss of consciousness				+	
Fever	+		+	+	+
Fall of blood pressure			+	+	

Patients 1 2 and 3 had co-existing pernicious anaemia Patient 5 had co-existing gastric anomaly and anaemia

TABLE II Dose of gammaglobulin followed by untoward reactions Time and duration of untoward reactions

	Time of untoward reactions	Dose that produced untoward reactions	Time of untoward reactions	Duration of untoward reactions
Case 1	November 1959	16 ml First 8 ml. Two hours later 8 ml	2—3 min	Some hours
	Autumn 1961	5 ml	2—3 min	Some hours
	Summer 1963	4 ml	2—3 min	Some hours
	March 1964	1.5 ml	During injection	Some hours
Case 2	May 1958	16 ml	1—2 min	About 10 min
	January 1960	16 ml	1—2 min	About 10 min
Case 3	December 1958	8 ml	5 min	1—2 hours
	June 1961	24 ml (8+8+8)	20 min	1—2 hours
	August 1961	24 ml First 2.5 ml Half an hour later 21.5 ml	20 min	1—2 hours
	August 1963[1]	*24 ml First 3 ml Half an hour later 21 ml*	*10 min*	1—2 hours
	November 1963[1]	24 ml First 3 ml Half an hour later 21 ml	5 min	1—2 hours
	April 1965[1]	10 ml	2—3 min	1—2 hours
Case 4	November 1961	8 ml	10—15 sec	1—2 hours
	February 1963[1]	8 ml	Some sec	1—2 hours
Case 5	After every monthly injection from October 1962 to September 1963	24 ml	10—12 hours	24 hours

[1] Gammaglobulin without merthiolate

TABLE III Number of uncomplicated injections and injections followed by untoward reactions

	Case 1	Case 2	Case 3	Case 4	Case 5
Number of injections followed by untoward reactions	4	2	6	2	11
Interval between beginning of treatment and first untoward reaction	2 5 years	1 year	5 months	3 years	5 years
Total volume of gammaglobulin given before first untoward reaction	640 ml	250 ml	120 ml	1 200 ml	250 ml
Number of injections before first untoward reaction	20	26	5	30—40	105
Number of uncomplicated injections between untoward reactions	18 12 6	40	17 1 29 2 21	20	0

i m and then 21 5 ml without any side effects

The same procedure was used for the next injection in August The patient was first given a dose of 2 5 ml and half an hour later 21 5 ml About 20 minutes after the second injection the patient reacted with nausea vomiting diarrhoea fall in blood pressure rigors and rise in temperature to 39 3 C A new intracutaneous test with gammaglobulin alone diluted 1 10 with physiological saline was negative Examination 12 days after the reaction revealed no antibodies to gammaglobulin From September 1961 the patient was treated with merthiolate free gammaglobulin 24 ml every 3rd—4th week The injections were given with the patient erect, and a test dose of 2—3 ml was given about 30 minutes before the rest of the dose

The injections produced no complications for about two years i e until August 1963 when 10 minutes after the second injection he reacted with feeling of general malaise cold sweat and fall in blood pressure The symptoms disappeared after some minutes without any special treatment About an hour and a half later the patient again felt worse with rigors and rise in temperature

In October 1963 nausea vomiting cold sweat and fall in blood pressure occurred about 5 *minutes after the second injection* Half an hour later a rigor developed and the body temperature rose

In April 1965 he had a new attack after having received an initial fraction of 10 ml as a test dose The injection was followed some minutes later by marked fall in blood pressure, nausea and faecal incontinence Neither the blood pressure nor the pulse could be recorded but the patient did not lose consciousness He was treated with adrenalin and steroids Some 10 minu es later he felt better but then rigors developed though they were not so severe this time as in August and November 1963 After about an hour he was again symptom free

Despite these side effects gammaglobulin treatment which was started in 1958 was continued but did not keep the patient free from infections He had purulent meningitis in September 1961 repeated respiratory tract infections for which he was admitted to hospital on three occasions in 1962—1964 and repeated intestinal infections He had

another attack of purulent meningitis in February 1965

Pernicious anaemia was diagnosed in January 1964

Case 4 A.A A man born in 1909, who had since 1931 had known pulmonary processes which alternately progressed and regressed Tuberculosis was strongly suspected but repeated cultures on Lowenstein s medium and guinea pig tests with the sputum bronchial washings and gastric washings regularly proved negative The tuberculin reaction varied in strength from one occasion to another from strongly positive to 0 05 mg to weakly positive to 1 mg Culture in 1938 confirmed tuberculous osteochondritis of the ribs of the left side and of the right scapula The condition was treated surgically at different times During the years 1949 to 1960 he had repeated respiratory tract infections pneumonia on about 40 occasions, and on 5 occasions Herpes zoster alternately on the right and left half of the chest In 1961 the patient was subjected to splenectomy in an attempt to improve the leukocyte response to infections Histological examination of the extirpated spleen showed a picture reminiscent mainly of that of sarcoidosis In 1962 he had colisepsis

Hypogammaglobulinaemia had been diagnosed since 1958 Serum electrophoresis that year showed a gammaglobulin value of 0 18 g/100 ml After 1958 he was treated with antibiotics periodically anticatarrhal vaccine and gammaglobulin in various doses Repeated electrophoretic examination of the serum showed gammaglobulin values ranging between 0 13 and 0 30 g 100 ml Blood group A Rh On three occasions in 1961 no anti B could be demonstrated During various spells in hospital the patient was sometimes treated with very large doses of gammaglobulin which was not followed by any side effects From March 30 to May 10 1961 he thus received more than 900 ml of gamma globulin intramuscularly

The first side effects occurred in November 1961 some 10—15 seconds after the end of an injection of 8 ml He reacted suddenly with malaise, vomiting urinary incontinence and transient loss of consciousness as well as severe cyanosis of the hands and feet feeling of tension in the chest tremor abdominal pain with frequent loose stools During the following hours the symptoms successively disappeared The body temperature rose to 37 8 C

Examination of the patient s serum one month after the side effect revealed no anti bodies to gammaglobulin Prausnitz Kustner s test on two occasions three weeks after the side effects proved negative In March 1962 intracutaneous and subcutaneous tests with small doses of gammaglobulin without merthiolate were negative and treatment was therefore continued with gammaglobulin without merthiolate 8—16 ml every 2 to 4 weeks

On February 4, 1963 the patient was given 8 ml and reacted immediately after the end of the injection with nausea, pronounced flush and pain in the chest and back Tachycardia was noted, but no fall in blood pressure The ECG was normal The symptoms stopped after about 1 hour but after a further half hour he had severe rigors The following day he felt tired but he was afebrile and was otherwise symptom free

The patient has since received prophylaxis with antibiotics alone and has been largely free from infections Gammaglobulin value in May 1965 0 21 g/100 ml

Case 5 L I A woman, born in 1922 During adolescence she had often had mild respiratory tract infections for which she had never been admitted to hospital From the age of 35 she had repeatedly had sinusitis and pneumonia In 1961 she had purulent meningitis Serum electrophoresis before treatment in 1957 showed total 6 0 albumin 4 1 α_1 globulin 0 31 α globulin 0 51 β_1 globulin 0 40 β_2 globulin 0 19 and gammaglobulin 0 51 g/100 ml *Blood group A Rh—* Anti B titre 1 32 During the following years the gammaglobulin concentration decreased in 1961 0 22, in 1963 0 19 in 1965 0 19 g 100 ml The anti B-titre in 1965 was 1 2

In 1957 treatment was started with 2 75 ml gammaglobulin every 3rd week, which however did not prove sufficient to keep the patient free from infections Examination in 1961 revealed impairment of appreciation of vibration and a gastric anomaly with severe stenosis of the pyloric canal Pernicious anaemia was suspected but the patient was not thoroughly examined for this condition Owing to the gastric anomaly she had a premature feeling of fulness at meals and absorption may have been impaired (albumin 3 25—2 86 g/100 ml during 1957—1963)

It is not known with certainty whether any side effects occurred when the patient was treated with gammaglobulin in a dose of 2 75 ml During that time she had repeated infections of the upper airways and fever and slight side effects if any of gammaglobulin treatment might have been difficult to distinguish from the patient s symptoms of infection In October 1962 after the dose had been increased to 24 ml once a month the 11 subsequent injections were regularly followed by side effects Ten to twelve hours after the injection she had incipient reactions with general malaise a feeling of tension in the chest lumbar pain pain in the limbs and pronounced dry cough and rigors with fever The symptoms often persisted for a day and were followed by marked tiredness for 3—4 days

Though the patient pointed out the connection between the symptoms and the injections the symptoms were interpreted by her physician as incipient infections of the upper airways and the patient was given antibiotics to counteract bacterial complications In 1963 the patient refused further injection of gammaglobulin and she then had no further attacks of such symptoms and her general physical condition improved

An intracutaneous test in December 1963 with 0 1 ml of undiluted gammaglobulin with merthiolate produced no reaction A dose of 2 ml of the gammaglobulin from the same batch intramuscularly was followed 10 hours later by malaise dry cough slight rigors and fever (38 4 C) The following day the patient was afebrile and symptom free There tes s were done while the patient was in hospital where she spent 14 days During this spell she was afebrile with the exception of the above mentioned rise of temperature

No antibodies to human gammaglobulin could be demonstrated in the patient s serum examined 2 days after the intramuscular test

Since September 1963 the patient has not received gammaglobulin She has sometimes had infections of the upper airways but has required hospitalisation for a short period on only one occasion In May 1965 she was admitted for investigation of progressive anaemia (Hb 54 % Red blood cell count 2 2 million MCV 122 MCHC 32 serum iron 33 γ % Haptoglobin 164 mg/100 ml Serum B 12 190 μg/ml Serum bilirubin 0 6 mg/100 ml Sternal puncture normal) Pernicious anaemia could thus not be confirmed

Subcutaneous tests (May 1965) with 0 2 ml undiluted gammaglobulin with and without merthiolate and with 2 ml in intramuscular tests with the same preparations were negative

Discussion

In cases 1—4 the symptoms were largely the same In these patients the untoward reactions occurred immediately after or within 20 minutes of the end of the injection and the side effects were relatively sporadic — most of the *injections of gammaglobulin* were not followed by any complications

Case 5 differed markedly from the other four The complications in the form of fever and dry cough did not appear until 10—12 hours after the injection and then regularly after every injection when the dose had been increased to 24 ml During observation in hospital however signs of untoward

reactions were demonstrated even after administration of a dose of 2 ml It would appear that the causal mechanism of the side effects in case 5 differed from that in the other cases For the time being case 5 is regarded as unique and will not be discussed further here

In an attempt to find out whether the injections of gammaglobulin produced side effects more often in patients with hypogammaglobulinaemia than in others, we analysed the records of a series of cases that had been treated at the department for infectious diseases in Lund From January 1, 1963 to June 30, 1964 more than 1,000 injections of gammaglobulin had been given intramuscularly, mainly prophylactically to largely healthy individuals Of these injections 240 were of 24 ml In no instance had any local or systemic symptoms been recorded Of all together 270 injections given in cases 1—4, fourteen were followed by complications The probability that none of the 1,000 doses in the former group, but 14 of 270 in the latter should have been injected intravenously is negligible There is thus hardly any reason to ascribe the symptoms in the patients with hypogammaglobulinaemia solely to accidental intravenous injection

An important difference between the patients with hypogammaglobulinaemia and the control series just mentioned is, of course, that the former received a series of injections of gammaglobulin, while the latter were rarely given more than one injection A question that arises is whether the reactions might have been due to some immunisation or sensitisation

It has been shown by serological methods that human gammaglobulin possesses group specificity, Gm and Inv-groups, which have been shown to be genetically determined (8, 9, 21) In the Swedish population about 60 % of all persons are Gm(a+) and thus 40 % Gm(a—)

It is now known that anti Gm(a) can develop in Gm(a—) individuals by exposure to Gm(a+) gammaglobulin This has been observed particularly in children (for review see Stiehm and Fudenberg (26)) Anti Gm(a) also often occurs during the first year of life in Gm(a—) infants of Gm(a+) mothers, the maternal gammaglobulin passing through the placenta and forming the stimulus (23, 25) Intentional immunisation of Gm(a—) volunteers with Gm(a+ gammaglobulin has also been reported (6) In 2 known cases transfusion of Gm(a+) gammaglobulin to individuals with anti Gm(a) has produced untoward reactions reminiscent of the ones observed in cases 1—4 (5, 6) As a rule, non haemolytic transfusion reactions cannot be attributed to this incompatibility

It is not possible to ascertain whether patients 1—4 are genetically Gm(a+) or Gm(a—), as family studies were not performed Patients with hypogammaglobulinaemia are phenotypically Gm (a—) unless they have received gammaglobulin, which is always Gm(a+) in serum pools

In none of the three cases examined could anti Gm or anti gammaglobulin be demonstrated in spite of the use of very sensitive methods, including testing with blood cells coated with anti D Ripley, a system known to be highly

sensitive The blood samples were taken 12 days or one month after the complication Thus nothing suggested that the reactions observed in our cases were due to immunisation against Gm determinants The irregular occurrence of the reactions also argues against immunisation

The gammaglobulin preparations used contain merthiolate as a preservative and traces of glycine, sodium carbonate, sodium acetate and saline The symptoms can hardly be ascribed to allergy to mercury, at least not in cases 3 and 4 where the reactions occurred after injection of gammaglobulin free from preservative No local reaction was seen at the site of the injection In addition, in 3 of the cases gammaglobulin preparations containing merthiolate which were given at various occasions after the injections followed by complications, were well tolerated

Another observation arguing against hypersensitivity was that in case 1 injection of 8 ml produced no complications while a further equal dose two hours later produced an untoward reaction within 2 minutes The sequence of events was roughly the same on 3 occasions in case 3 in which 2 5 ml or 3 ml was given first and half an hour later an injection of about 20 ml The side effects appeared 5—20 minutes after the latter

It would thus appear that patients with hypogammaglobulinaemia are more inclined than others to react unfavourably to intramuscular administration of gammaglobulin In our opinion the data do not favour the interpretation that these capricious reactions are attributable to immunisation, allergy or accidental intravenous injection

In a study on 70 persons who were given gammaglobulin intravenously under standardized conditions Barandun et al (2, 3) found that the side effects varied with the anti complementary activity of the preparations used and that persons with hypogammaglobulinaemia were more sensitive and showed a much higher frequency of side reactions (93 3 %) than the controls (12 7 %) In some cases the reactions could be suppressed by the use of diluted gamma globulin or of a longer infusion period On the other hand, side effects could sometimes be provoked in normals by infusion of undiluted (16 %) solution Antagonists to histamine and serotonin had no significant influence on the side effects

The untoward reactions in the abovementioned series reported by Barandun et al were sometimes biphasic A biphasic course was more typical of mild reactions the initial flush, feeling of tension in the chest, dyspnoea and lumbar pain disappearing before the later development of rigors and fever In the more severe reactions resembling anaphylactic shock and sometimes associated with a marked fall in blood pressure and loss of consciousness the initial symptoms were followed immediately by rigors and rise in temperature Even very severe reactions usually disappeared within 3—4 hours

The above mentioned untoward reactions to intravenous administration of certain gammaglobulin preparations thus closely resemble those observed in our patients 1—4 who had received gamma

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The Clinical Manifestations of Temporal Arteritis

By

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Temporal arteritis is considered to be a rare disease being at any rate infrequently diagnosed This is due in part to the fact that the patients, who are about 70 years of age, often are suspected of malignancy or severe infection on account of their general symptoms, e g fever anorexia weariness, weight loss and increased erythrocyte sedimentation rate Attention is therefore mostly focussed on roentgenological and laboratory investigations, and inconspicuous and atypical local symptoms in the temporal region may easily escape recognition Diffuse pain and tenderness in the scalp and pain in the back of the head are often the dominant symptoms

Apart from local symptoms over the temporal artery, which on palpation proves to be hard, swollen tortuous and in advanced cases has ceased to pulsate disturbances of vision sometimes leading to blindness, and various psychic symptoms are the commonest manifestations of the disease

Temporal arteritis was first described by Horton et al in 1932 (18) In typical cases the histological finding consists of a granulomatous inflammation of the media and intima, which sometimes exhibit necrotic foci The lamina elastica interna may be markedly tortuous and in part fragmentated Multinuclear giant cells are often encountered in its vicinity The vascular lumen is entirely or partially obliterated by the proliferating intima In large series cases have been described which showed the clinical features typical of temporal arteritis, although biopsy specimens exhibited non specific arteritis without giant cells (28)

Extracranial manifestations of the disease in the aorta and its branches the subclavian and external carotid arteries, and in the coronary, iliac splenic and renal arteries were first described by Gilmour in 1941 (13) and Cooke et al (8) later by Cardell and Hanley (7) and Heptinstall et al (17) The symptoms originate in obliterating arteritis of the respective organs

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TABLE I Clinical data

Case no	Sex	Age	Duration of disease on admission	Headache: Typical temporal signs	Headache: Other localization
1	♀	63	3 weeks	Typical local signs No pain	Occipital Painful scalp
2	♀	70	5 weeks	Positive	Occipital
3	♀	76	3—4 months	Positive	Occipital
4	♂	70	2 months	Positive	Occipital
5	♀	64	3—4 months	Slight temporal pain	Occipital
6	♂	66	5 months	Positive	Painful scalp
7	♀	66	6 weeks	Positive	Occipital

leading to ischaemic lesions In addition, aneurysms of the aorta and its main branches have been reported in cases of giant cell arteritis with or without temporal symptoms (15, 21, 24)

The various organ manifestations in giant-cell arteritis were described by Paulley et al (28)

Recently the term polymyalgia arteritica has been applied to a special syndrome, perhaps a form of disseminated giant cell arteritis frequently exhibiting symptoms from the temporal artery In particular, this term has been used by Swedish authors (14 15) Previously, the designation polymyalgia rheumatica was used (2, 5) The disease which affects elderly people, is characterized by acute muscular pain, stiffness and pain in different joints without effusion, and by increased erythrocyte sedimentation rate Temporal arteritis is frequent in different stages of the disease In many cases biopsy specimens from the temporal artery have exhibited giant cell arteritis or non specific arteritis although temporal symptoms were absent Hence, it has been assumed that the underlying cause of the muscular symptoms is an arteritis of the kind indicated by the name of the disease (2 14, 15)

The aetiology of giant cell arteritis and of the non specific arteritis in polymyalgia arteritica and temporal arteritis is entirely unknown Many authors regard these disorders as belonging to the group of auto immune diseases (12 24), and the relationship with other forms of necrotizing angiitis of

Muscular and joint pain	Eye symptoms Ophthalmological findings	Psychic symptoms
—	No symptoms Normal findings	Dementia confusion
Effusion in left knee	Subj impaired vision Normal findings	Slight confusion
—	Blurring of vision Senile cataract Other findings normal	Dementia
—	Impaired vision Perivascular oedema in both temporal quadr Visual fields normal	Depression
Shoulder stiffness chewing pain dysphagia migrating joint pain	Normal findings	Emotional lability
—	Normal findings	—
—	Slight arterial calibre variations in eye grounds	—

admittedly auto immune aetiology e g polyarteritis nodosa, is often emphasized (1)

In the present paper 7 cases of temporal arteritis, verified by biopsy are described particular attention being directed to the various extracranial manifestations of the disease and to the relationship between the latter and polymyalgia arteritica

Material

The series consists of 7 cases of temporal arteritis 5 women and 2 men The diagnosis was in all cases verified by biopsy specimens taken from a branch of one of the temporal arteries Two of these patients (nos 6 and 7) have previously been described (11) The mean age in the present series was 67 years Five patients were followed up for one to two years the remaining 2 have not been examined since they recovered

Table 1 shows the main clinical symptoms and signs and the duration of the disease prior to admission to the hospital Six patients were referred to the hospital on account of poor general condition fever of unknown origin and increased erythrocyte sedimentation rate Of these 3 had shortly before been examined in another hospital on account of the same symptoms without a correct diagnosis being made They had been examined mainly for malignancy or collagenous disease According to the hospital records no palpation of the temporal arteries had been made in 2 of the cases and in the third case a pulseless temporal artery had been taken for a thrombosed vein In one case only had the remitting physician suspected the presence of temporal arteritis on the basis of the local findings alone

The clinical picture was very much the same in all cases In addition to weariness weight loss and ation of temperature to

TABLE II Laboratory, biopsy and urographic findings

Case no	Erythrocyte sedim rate (mm/hr)		Paper electrophoresis of serum proteins			
				Globulins (per cent of total proteins)		
	Before treatment	After treatment	Total protein (g/100 ml)	α_2	γ	Rheumatoid factor
1	115	18	6 6	16 8	13 9	Negat
2	120	80 (1 week)	6 4	22 8	19 3	Negat
3	124	40	7 0	15 0	33 9	Negat
4	73	16	6 2	13 7	23 1	Negat
5	125	16	7 0	19 4	24 3	Negat
6	92	35	8 1	12 1	24 0	Negat
7	81	38	6 8	15 1	23 6	—
Mean values	104	35	6 9	17 9	23 2	

[1] Normal on first admission See case report
[2] See case report

between 37 and 39 C all patients had some kind of headache In 5 cases the pain was localized to the region of the temples where tender, tortuous and sometimes entirely pulseless temporal arteries could be palpated *In the remaining 2 cases* the discomfort was more vague and manifested itself as pain in the back of the head and tenderness over the whole of the scalp which on palpation exhibited tender nodules in one case These symptoms also occurred in conjunction with temporal symptoms

Disseminated muscular symptoms and anarthritic joint symptoms resembling those seen in polymyalgia arteritica were observed in only one case (no 5) Another patient (no 2) showed swelling and effusion in one knee joint due mainly to deforming arthrosis

Psychic symptoms were strikingly frequent Among these, impaired memory depression and progressing senile dementia were the commonest In one case acute disorientation developed though not to a degree necessitating admission to a mental hospital

Four of the patients had subjectively impaired vision or blurring of vision during some stage of their illness but no serious complications occurred such as diplopia marked limitation of the visual fields or visual acuity, or blindness *which is the most frequent* complication in this disease Six patients were examined by an ophthalmologist who measured visual acuity and the visual fields and performed ophthalmoscopy In one case only (no 4) ophthalmoscopy revealed perivascular oedemata in both temporal quadrants, frequently situated in formations resembling clusters of grapes

Nothing pathological was observed in the heart or lungs of any of the present patients nor hypertension with elevation of the diastolic pressure The electrocardiogram was normal in all cases

Table II shows the most important results of the laboratory and roentgenological investigations and the biopsy findings

Prior to the institution of treatment all patients had a markedly increased erythrocyte sedimentation rate (ESR), varying between

LE-cell test	Urine	Serum creatinine (mg/100 ml)	Urography	Biopsy of temporal arteries
Negat.	Norm	0.7	Normal	n.s.a.
Negat.	Norm	0.8	Susp. tumour in right kidney Normal angiography	n.s.a.
Negat.	Norm	0.7	—	g.c.a.
Negat.	Norm [1]	[1]0.9	Normal[1]	g.c.a.
Negat.	Norm	0.7	Susp. tumour in right kidney[2]	n.s.a.
Negat.	Haematuria (1 specimen)	1.0	Normal	g.c.a.
Negat.	Norm	0.7	—	n.s.a.
		0.78		

n.s.a. = non specific arteritis
g.c.a. = giant cell arteritis

73 and 124 mm/hr. As a rule, the ESR was normalized during two to four weeks of corticosteroid treatment and the clinical symptoms mostly disappeared as soon as within a few days. In case 2 the ESR was checked one week after the institution of treatment and no later check-ups were made.

Slightly elevated gamma globulin values were noted in the majority of the cases and all patients exhibited markedly elevated alpha globulin levels and hypoalbuminaemia. This finding is consistent with the electrophoretic pattern in acute infection. Highly elevated $alpha_2$ globulin and fibrinogen values and increased ESR are typical of temporal arteritis. In 3 cases (nos. 1, 3 and 5) immunoelectrophoresis was performed, which showed enhanced lines in the alpha region consisting mainly of haptoglobulins, beta A globulins and gamma-globulins.

All patients had normochrome anaemia with Hb values between 9 and 11 g per 100 ml and a normal leukocyte count with normal differentiation. None of the patients showed any signs of rheumatoid arthritis or lupus erythematosus disseminatus (LED); they all responded negatively to tests for rheumatoid factor, LE cells and antibodies to nucleoprotein.

As judged by examination of the urinary sedimentation and determination of the serum creatinine, the urine and the renal function were initially normal except in case 6, in which microscopic haematuria without proteinuria was observed in a single urine specimen. Unfortunately no check-ups were made and the cause of the haematuria thus remained obscure. The urographic findings were normal too. In case 4 haematuria and acute renal failure occurred in connection with an attack due to renal calculi four months after the institution of treatment. Three weeks later a concretion was removed from the right ureter. This case is briefly described below.

In cases 2 and 5 the kidneys were examined by angiography on account of abnormal urograms suggestive of a renal tumour. The renal findings were normal in both cases.

but in case 5 an aneurysm was detected in the lower part of the aorta immediately above the bifurcation The case is described below

Biopsy findings

A branch of one of the temporal arteries was resected for histological examination in all cases On the basis of the presence or absence of multinucleated giant cells the patients were divided into two groups Those without giant cells are designated the non specific arteritis group In 3 cases (table II) giant cells were observed usually close to the lamina elastica interna which in parts was tortuous and fragmentated Apart from the presence of giant cells, this form of arteritis could not be histologically distinguished from the non specific arteritis shown by the remainder of the patients All biopsy specimens exhibited a markedly thickened intima, entirely or partially obliterating the vascular lumen and inflammation with necroses and formation of connective tissue in all layers of the vascular wall in the media in particular Fragmentation of the lamina elastica interna was seen in parts Among the infiltrating cells lymphocytes and fibroblasts were dominant

Case reports

Case 4

First admission A 70 year-old male engineer was admitted to the hospital in December 1963 on account of severe bilateral pain in the temples of two months' duration The remitting physician had suspected temporal arteritis owing to the presence of hard tender and tortuous temporal arteries The patient exhibited the clinical features typical of this disease A biopsy specimen from a branch of the left temporal artery showed arteritis with a few giant cells The thickened intima occluded almost the entire vascular lumen and the media exhibited round-cell infiltrations in abundance The most important of the other data may be seen in tables I and II

Between 1951 and 1956 the patient had had several attacks caused by renal calculi and in 1956 a large solitary concretion had been removed from the left renal pelvis Subsequently no similar attacks had occurred During the present hospitalization serum creatinine the urinary sedimentation and the urographic findings were normal as is seen in the tables The patient became symptom free after prednisolone therapy consisting of 20 mg a day The disturbances of vision and psychic symptoms noted on admission also disappeared in a few weeks time The patient was discharged on Dec 17 1963 with a prescription of 15 mg prednisolone every second day

Second admission On account of haematuria without any other symptoms the patient was readmitted on March 5 1964 He exhibited no temporal symptoms The ESR was 16 mm/hr

The urographic findings were normal and the serum creatinine was 1 1 mg per 100 ml Cystoscopy revealed an enlarged prostate markedly protruding into the bladder Since the surface was bleeding this was assumed to be the source of the haemorrhage

Third admission On March 19, 1964 the patient was admitted again to the hospital on account of violent pain in the right flank which aroused the suspicion of a calculus in the right ureter This time the serum creatinine value was initially 1 8 mg per 100 ml and rose to 2 1 mg per 100 ml on March 23 During the first few days no oliguria was observed but on the day of admission there had been copious vomiting In spite of a satisfactory urinary output of 1 600—2 400 ml daily the serum creatinine remained for two weeks on a level of about 2 2 mg per 100 ml The specific gravity of the urine was initially under 1 010 Hence the suspicion of acute tubular necrosis due to the acute attack of pain was maintained Urography was performed on the fifth and ninth days after admission but no calculi were demonstrable in the urinary tract However delayed cumulation and delayed elimination



Fig 1 Case 4 Needle biopsy specimen from the left kidney Note the extensive interstitial fibrosis van Gieson staining



Fig 2 Case 5 Aortography showing normal kidneys normal renal arteries and an aneurysm of the aorta

of the contrast medium in the right kidney slight hydronephrosis and poor visualization of the whole ureter were indicative of an invisible obstacle in the proximal portion of the right ureter No destruction of the renal parenchyma was observable on either side On April 5 a small calcium oxalate concretion was removed from the right ureter via the bladder The haematuria disappeared but the creatinine was not back to normal until three months later During this time prednisolone medication of 15 mg every second day was given An uncomplicated prostatectomy was performed in February 1965 The creatinine was then 1 1 mg per 100 ml the ESR was 13 mm/hr and no haematuria was present Renal biopsy was unfortunately not performed during the period of acute renal failure On Oct 11 1965 a successful biopsy was performed on the left side The patient had then been free from temporal symptoms but had had intermittent pain in the region of the left kidney The prednisolone therapy (5 mg daily) had been discontinued two weeks before The serum creatinine was then 1 25 mg per 100 ml and the ESR was 10 mm/hr

Microscopic examination revealed extensive interstitial fibrosis in the whole biopsy specimen which consisted of both medullary and cortical tissue (fig 1) Unfortunately it contained no glomeruli No inflammatory cells were observed A few small arterioles without inflammatory changes were present The tubuli were in part atrophic

Case 5

A 63 year old married woman was admitted on April 5 1964 on account of fever weariness loss of weight headache and migrating muscular and joint pain of three weeks duration The whole of the neck the muscles in particular were very tender on palpation Chewing and swallowing were painful In addition she experienced pain in the shoulder joints and the muscles of the neck The temporal arteries were definitely tortuous and tender but pulsations were still felt in both A biopsy specimen from a small branch of the right temporal artery showed obvious inflammation of all layers of the wall but no giant cells The remainder of the most important examinations are presented in tables I and II The electrocardiogram was completely normal the blood pressure was 140 / 170 mm Hg The heart and lungs were normal both on auscultation and roentgenographically

The patient exhibited no clinical signs of rheumatoid arthritis Tests for rheumatoid factor and LE cells were negative No destructive changes were revealed by roentgenographic investigation of the skull thoracic

spine, pelvis and extremities The Hb was initially 9 1 g per 100 ml the erythrocyte count was 3 35 million per mm³, the mean erythrocyte diameter was 29 the leukocyte count was 11,000 per mm³ with normal differentiation Urography revealed slight bulging of the lower pole of the left kidney Hence renal angiography was performed This showed no signs of renal tumour but in the lower portion of the aorta above the bifurcation an aneurysm like protuberance with uneven wall contours was seen Furthermore, the course of the aorta as far as the renal arteries was highly tortuous and the outlines of the walls were irregular (fig 1)

With a prednisolone therapy of 20—30 mg a day the patient became asymptomatic in two weeks during which the ESR dropped to 30 mm / hr She has remained symptom free with a maintenance dose of 5—10 mg prednisolone every second day

Discussion

The classic temporal arteritis is today regarded as a local variation of the disseminated arteritis lately designated polymyalgia arteritica (14, 15), previously known under the name poly myalgia rheumatica (2, 5, 29) In this syndrome the primary lesion is believed to be a giant cell arteritis, but non specific arteritis is frequently seen in clinically identical cases (15 28) Owing to the high mean age of the patients, the term senile arteritis has also been suggested (26 27)

In the present series it is only case 5 that can be regarded as a typical instance of polymyalgia arteritica with concurrent temporal symptoms, corresponding to the case reports of previous authors (14, 15) The pain and tenderness in the muscles of the neck and masticatory apparatus and in the muscles of the extremities were attributed to an arteritis of the same kind as was present in the temporal artery In Alestig and Barr s series (2) case 5 exhibited similar features, including chewing pain

Pain in the neck was strikingly common in the remainder of the present patients, too, suggesting arteritis of the occipital artery and its branches in the musculature of the neck Such cases may be regarded as typical instances of temporal arteritis with features in common with polymyalgia arteritica

It seems to be a tenable assumption that polymyalgia arteritica is a relatively frequent disease, although numerous cases remain undiagnosed owing to the fact that its general symptoms, including vague joint and muscular pain, are a common accompaniment of old age Hamrin et al (15) described 52 cases, diagnosed during a period of four years Of these patients 18 had typical temporal arteritis during some stage of their illness In addition, on the basis of a routine biopsy specimen arteritis of the temporal artery was detected in 11 patients who had no headache

It appears that the large arteries are relatively often affected by the disease In 30 of Hamrin's patients murmurs over large arteries were heard on auscultation In 5 cases the findings were angiographically verified as vascular stenosis, presumably due to the arteritis observed in biopsy specimens from the temporal artery In the series in question no vascular aneurysms were observed (15)

Vascular aneurysms very rarely occur in association with giant cell arteritis and appear mainly to affect the aorta

and its major branches (16) According to Harrison (16), this is due to the fact that in larger vessels the intima does not proliferate inwards sufficiently to obliterate the lumen as it does in minor vessels Instead, the affected media yields to the intra arterial pressure and an aneurysm develops In case 5 in the present series the patient had an aneurysm of the aorta above the bifurcation The aorta was highly tortuous with an uneven wall contour which might well be attributed to disseminated arteritic changes Otherwise, the patient did not clinically appear to be sclerotic Apart from this aneurysm no other manifestations of the disease were observable in large vessels Systematic investigations on this point were not performed however

In autopsy material, giant cell arteritis has been observed in almost all the main branches of the aorta (7) As early as 1938, Jennings (20) described the aortic arch syndrome associated with temporal arteritis This syndrome has since been reported in polymyalgia arteritica (2 14 28) Furthermore, Ask Upmark (3) suggested that there may be a close connection between giant cell arteritis and the arteritis of Takayashu s syndrome It may perhaps be assumed that both the typical temporal arteritis and the aortic arch syndrome are extreme local variations of the same generalized vascular disease which manifests itself clinically as polymyalgia arteritica The classic temporal arteritis just as for polymyalgia arteritica almost invariably occurs in middle aged and elderly people as was the case in the present series, too Only one case of giant cell arteritis with temporal symptoms verified by biopsy has been described in young person viz in a 36 year-old man (6)

The connection between the arteritis of old age and the arteritis of the aorta and its branches which almost exclusively affects young people mostly young women is obscure Schrire (30) has recently discussed the arteritis occurring in young women which usually affects the brachiocephalic trunk, and as a rule manifests itself clinically as Takayashu s syndrome On the basis of the histological findings he describes this arteritis as "diffuse" and accords no significance to the sporadic occurrence of giant cells

It has been assumed that both these forms of arteritis are auto immune diseases (12 24), but the obvious predisposition of certain age groups to one or the other is difficult to explain

Apart from the presence of giant cells in the biopsy specimens from the temporal arteries in three of the present cases, these could not be distinguished histologically from the remaining four cases, which exhibited non specific arteritis The two groups likewise did not differ clinically Similar observations have been reported in larger series (14 28)

The significance of the giant cells is obscure According to Isaacson (19) giant cells as a rule occur in sites showing degeneration of elastic tissue This is consistent with the histological picture in temporal arteritis where giant cells are found close to the often fragmentated and degenerated lamina elastica interna Eulefeld (12) regards the changes in the elastica as the primary lesion in giant cell arteritis and assumes that the

and joint pain, which is a feature typical of polymyalgia arteritica Furthermore she had an aneurysm of the aorta, which was regarded as a manifestation of the same disseminated arteritis

Pathological urinary findings and renal failure of a glomerulotubular nature are surprisingly seldom reported in temporal arteritis It may assumed, however, that such complications are not infrequent, considering that the disorder in question is a generalized vascular disease, like polyarteritis nodosa A case showing acute renal failure, possibly due to an interstitial nephritis concurrent with the temporal arteritis is described The conclusion is drawn that renal biopsy ought to be performed more often, and during the acute stage of the disease

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On the Influence of Body Position on Steady-state Diffusing Capacity during Exercise, Studied in Patients with Pulmonary Sarcoidosis

By

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In earlier studies (16, 33, 34) on the steady state diffusing capacity of the lungs for carbon monoxide, D_{LCO}, in patients with pulmonary sarcoidosis, a low D_{LCO} was observed in all stages of the disease This was the case even in patients who only had hilar lymphomas without parenchymal infiltrations In all stages there was also a higher than normal incidence of orthostatic increases in heart rate at rest in standing position and during exercise in sitting position The investigators (16) suggested that the orthostatic shifts within the capacitance vessels, indicated by these increases in heart rate (9), might also cause a decrease in the pulmonary capillary blood volume and thereby also D_{LCO} Such decreases in D_{LCO} have been reported for resting subjects shifting from supine to standing position by a number of investigators (3 21, 22)

On the basis of the normal regression between D_{LCO} during exercise at heart rates above 120 beat per minute and total hemoglobin (THb) (12), the material reported earlier (16, 33 34) was analyzed with regard to whether a marked postural increase in heart rate at rest or during exercise was accompanied by low D_{LCO} during exercise in the sitting position (fig 1) The figure shows that there was no obvious correlation between low D_{LCO} and marked orthostatic heart rate increases in these patients with sarcoidosis

The present investigation was undertaken to verify this analyses and to eliminate postural blood shifts as a possible cause of low D_{LCO} in sarcoidosis

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Methods and material

The methods used in the present investigation and the errors involved in determination of steady state D_{LCO} have been reported earlier Only significant data will be given here (16, 17)

Orthostatic test An orthostatic test was performed in each subject, consisting of recording of the heart rate and ECG at rest and after eight minutes standing in the erect position

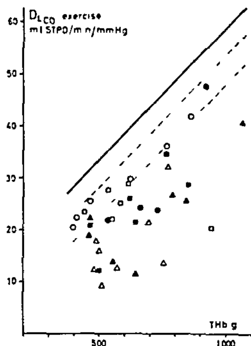


Fig 1 Relationship between D_{LCO} during exercise ml STPD min⁻¹ mm Hg⁻¹ and total hemoglobin THb g in 11 patients with bilateral hilar lymph node enlargement (16) circles 11 patients with parenchymal infiltrations (33) squares and 15 patients with fibrosis of the lungs (34) triangles Open symbols indicate patients with heart rate in standing position of ≥ 110 beats per min and/or heart rate that was ≥ 10 beats per min higher in sitting than in supine position

Exercise tolerance test The rate of work that the patients could perform at a heart rate of 170 beats min W_{170} kpm/min (31) was determined in each patient both sitting and supine on an electrically braked ergometer (9)

Static lung volumes (l BTPS) Total lung capacity with its subdivisions were determined by the helium dilution method with a closed spirometer system (14) Normal values were predicted from nomograms (13) *Dynamic lung volumes* were measured with a modified Bernstein spirometer (Kifa) (8) Normal values were predicted from the nomograms published by Berglund et al (6)

Distribution of inspired gas was studied with the aid of N_2 elimination during oxygen breathing use being made of the Lilly N_2 meter as modified by Lundin (26) The result was expressed as the time, min and total V_E, l, for lowering of F_{EN_2} to 0 02, divided by FRC determined as the F_{EN_2} = 0 02 Nitrogen space' (4)

Venous admixture due to true shunts in the lungs was estimated as outlined by Berggren (5) The patients breathed 100 per cent oxygen until nitrogen was practically washed out of the lungs as judged from the N_2 wash-out curve For further methodological details see also Holmgren and Svanborg (16)

Fractions of gases F_{O_2} and F_{CO_2} were analyzed by the Scholander technique (28) *Gas volumes* were measured with a spirometer, (32) *Carbon monoxide* in the gas phase was analyzed with a hopcalite CO meter (Stålex) (24) *Carbon monoxide in blood* was determined by the method described by Linderholm et al (25) The CO meter was calibrated with carefully prepared mixtures of CO in air *The partial pressure of CO_2 in arterial blood,* P_{aCO_2} mm Hg was measured with a P_{CO_2} electrode (30) and with the micro-technique described by Andersen et al (1) For alveolar gas exchange only data obtained with the Severinghaus electrode (16 17) were used *pH and standard bicarbonate* were determined with a micro glass electrode (1)

Symbols were those suggested by Pappenheimer et al (27)

Calculations The steady state D_{LCO} of the lungs was calculated according to Filley (11) with use of the correction for CO back pressure suggested by Linderholm (23)

Methodological errors have been reported separately (15) All computations were performed with an IBM 1401 computer

Material and procedure

The material consists of 12 patients with pulmonary sarcoidosis 10 women and two men aged from 28—49 years Eight of the women had bilateral hilar lymphomas only (group I) (16 33 34) Case 1 had minor parenchymal infiltrations (group II) and case 8 had minor infiltrations plus roent

genological signs of fibrosis (group III). One of the men had hilar lymphomas only and the other, case 7, minor parenchymal infiltrations and possibly fibrosis of the lungs.

The diagnoses were supported by lymph-node biopsy findings in all cases.

The case histories were similar to those earlier reported (16, 33, 34) with dyspnoea, orthostatic complaints and tiredness as major symptoms.

The subjects arrived at the laboratory early in the morning and all had a standardized light meal. A teflon catheter was introduced into the left brachial artery with a percutaneous technique (7) and was left there during the examination.

The first study was performed alternately in sitting and supine position. The load used in the study should increase the heart rate to between 130—150 beats per minute and was selected with the aid of work tests already performed in the sitting and supine positions for determination of W_{170}. Only one load was studied in each patient. During each work period the subject breathed without mouthpiece for the first four minutes and then air through a low resistance air collection system for one minute. 0.05 per cent CO in air for two minutes after which period a steady state of the CO uptake was reckoned to be present (2). Expired air was then collected for two minutes in a Douglas bag. During the first minute an arterial blood sample was withdrawn slowly for determination of gas tensions and pH. In the middle of the air collection period another blood sample was withdrawn for determination of CO saturation and hemoglobin concentration. This value for S_{CO} was regarded as a mean value for the sampling period. Between the two work periods the patients rested for half an hour. All determinations of gas tensions were performed immediately and corrected for the difference between rectal temperature and cuvette temperature (19).

Results

Anthropometric data are presented in table I.

Heart volume in the supine position was determined in 9 patients. In case 2 the films could not be used for the calculation and in case 1 and 3 no X rays were taken. Heart volume fell within the normal range of variation in relation to body size in these 9 patients (15).

Orthostatic test. The heart rate increased above 100 beats per minute — which is considered to be the upper limit of a normal range of variation (15) — in cases 3, 6, 7, 10 and 11, i.e. in five of 11 patients investigated.

Exercise tolerance. W_{170} in sitting position fell within the normal range of variation in relation to sex, body weight and heart volume (15). However, in the supine position there was an increase in W_{170} by an average of 150 kpm/min in 7 patients, a decrease in one and no difference in three patients. The same tendency to an orthostatic increase during exercise is also illustrated by the heart rates at the same load in sitting and supine positions in table I.

Static lung volumes. Vital capacity averaged 103 per cent of the predicted normal value (range 66—139), *FVC*, 102 per cent (range 66—147) and *total lung capacity* 99 per cent (range 62—132). The *residual quotient* averaged 26.6 per cent (range 21—35).

Nitrogen wash-out time averaged 2.0 min (range 0.9—2.8), i.e. all patients fell within the normal range of variation. The *nitrogen wash-out index* averaged 6.67 (range 4.0—8.1), also within the normal range of variation.

The *venous admixture* at rest (table II) averaged 5.3 per cent of the pulmonary capillary blood flow, (range 3.6—9.7). If values above 5.2 per cent are taken

TABLE I Anthropometric data and results from exercise tolerance test in sitting and supine positions W_{170}, kpm out VC = vital capacity FRC = functional residual capacity TC = total lung capacity RV = residual volume in one second FEV % FEV 1 0 in per cent of the forced vital capacity MMF = maximal mi

Sex	Group	Age (yrs)	Height (cm)	Weight (kg)	Heart vol (ml)	Hb conc (g/100 ml)	Pulse rate standing (beats/min)	W_{170} sitting (kpm/min)	W_{170} supine (kpm/min)	Orthostatic test pulse rate at work: Load (kpm/min)	Sitting	Supine	VC: Obs l BTPS	% of predicted
♀	II	30	159	48	–	15 1	–	–	–	300	146	131	2 90	101
♀	I	46	160	71	–	14 7	88	500	650	400	148	132	2 48	83
♂	I	28	177	73 6	–	15 2	110	1 000	1 300	900	158	131	6 07	113
♀	I	41	156 5	56	710	14 4	85	~700	850	600	148	142	3 40	110
♀	I	40	159 5	77 8	790	13 4	80	650	650	600	165	162	3 58	119
♀	I	32	168	58 5	560	13 6	110	~600	600	600	167	162	4 83	139
♂	II(III)	49	170	55	655	16 7	100	550	450	300	140	150	2.89	76
♀	III	37	174 5	77 5	765	14 2	88	600	750	600	168	153	4 06	101
♀	I	43	162 5	61	630	13 8	73	900	950	800	163	158	3 41	103
♂	I	32	172 5	74	730	15 3	109	>1 000	>1 000	600	130	125	4 32	111
♀	I	29	166	69	160	14 5	114	550	650	600	175	163	3 13	89
♂	I	37	187	89 5	1 000	–	78	1,200	1 350	1 200	168	153	5 30	9[illegible]

as the upper normal range, a significant shunt can be regarded as present in cases 9, 10 and 11

Results of measurements during exercise in sitting and supine positions

Primary data and a statistical comparison between data obtained in supine and sitting are presented in tables II and III

In the sitting position *the heart rate* was significantly higher, by 9 5 beats at a level of 150 9 beats per minute *Ventilation* of the lungs was effected with the same tidal volume and respiratory rate in both body positions *Oxygen uptake* was however slightly higher in sitting position but not significantly so The *physiological dead space* was of the same order in both body positions.

Arterial oxygen tension averaged 90 9 mm Hg in the supine position and did not change significantly during exercise in the sitting position P_{aCO_2} averaged 34 8 mm Hg in the supine position and remained the same during exercise in the sitting position *pH* was slightly higher by 0 013 mm Hg, in the sitting position, but standard bicarbonate remained unchanged *RQ* was significantly lower in the sitting position

Pulmonary diffusing capacity averaged 29 2 during exercise in the supine position In 7/12 patients the difference sitting—supine was positive, i e D_L was higher in the sitting position In two patients the results were identical and in three $D_{I_{CO}}$ was higher in the supine position The mean difference was 0 6 units higher in the sitting than in the

m n stat c and dynamic lung volumes 1 BTPS and intrapulmonary gas m xing studies with nitrogen was volume $MVV_{f\,ee}$ maximal volontary ventilation at a free resp ratory rate $FEV_{1\,0}$ forced exp rator exp ratory flo v The mater al cons sts of 12 pat ents w th sarco dos s of the lungs

FRC		TC		$\frac{RV}{TC} \times 100$	$MVV_{f\,ee}$			$FEV_{1\,0}$		FEV %		MMF		
Obs 1 BTPS	% of pred cted	Obs 1 BTPS	% of pred cted	Obs %	Rate	Obs (1/m n)	% of pred cted	Obs 1	% of pred cted	Obs %	% of pred cted	Obs (1/sec)	N_2 wash o t t me (m)	$\frac{VE}{VRC}$ ($I\,FN_2$ − 2 %)
2 38	104	4 46	99	35	60	50	47	2 01	87	73	87	1 86		
1 74	102	3 43	78	28	46	67	65	2 00	74	74	96	1 57	2 7	7 8
3 32	95	7 61	114	21	36	120	68	4 12	98	66	82	3 40	2 0	5 8
2 13	107	4 54	111	25	42	58	54	2 82	104	87	106	3 26	1 8	4 9
2 20	147	4 72	110	24	40	100	94	2 99	107	85	104	3 67	0 9	4 0
3 21	128	6 58	132	26	40	122	107	3 90	122	76	91	3 24	1 4	8 1
2 58	66	4 02	62	30	48	55	40	1 87	58	70	95	1 66	1 7	8 1
3 15	93	5 22	97	25	42	111	101	3 30	100	83	101	3 44	2 1	6 2
2 05	98	4 21	94	28	48	102	97	3 08	110	86	106	4 32	2 4	7 9
1 97	86	5 06	96	21	50	135	118	3 69	109	86	102	4 42	2 2	7 4
2 10	100	4 40	90	31	38	71	61	2 51	76	79	93	2 27	2 8	6 4
3 35	99	7 00	99	25	39	160	93	4 45	106	82	106	5 11	2 4	6 8

supine position but the difference was not significant

In earlier studies (12) the relation sh p between D_{LCO} during exercise at heart rates ≥ 120 beats per minute was found to be highly correlated to a number of body parameters such as body height heart volume W_{170} and vital capacity When these two materials were analyzed together high regressions were found between D_{LCO} and $W_{1\,0}$ ($r - 0\,86$) VC ($r - 0\,84$) The regres sion equation for W_{170} was

$D_{LCO} - 0\,0221\ W_{170} + 17\,2$

S D $- \pm 4\,6$ $n - 38$

and for vital capacity

$D_{LCO} - 7\,130\ VC + 5\,4$

S D $= \pm 4\,8$ $n = 38$

The present data and those of Holm gren and Svanborg (16) are compared with these regressions in figs 2 and 3 A slightly low D_{LCO} in the sitting posi tion (< − SD) when related to VC was present in 4/12 patients (fig 2) cases 7 3 6 and 10 Of these four case 7 had a group III type of sarcoido sis In case 3 D_{LCO} was markedly higher in the supine than in the sitting position (6 units) This patient also had higher $W_{1\,0}$ (300 kpm/min) in the supine posi tion and a lower heart rate during exer cise when supine (table I) In case 6 there was a moderate increase of D_{LCO} when supine (3 units) and in cases 7 and 10 D_{LCO} was higher when sitting

Neither of these patients had signs (table I) indicative of significant blood shifts during exercise Of the 8 patients with normal D_{LCO} in relation to VC a marked increase when supine was

TABLE II Respiratory data during moderate exercise in sitting and supine positions in 12 patients

Case no	Work load (kpm/min)	Heart rate (beats/min)	Respiratory rate (breaths/min)	$\dot{V}_E$ (l BTPS/min)	V_T (ml BTPS)	$\dot{V}_{O_2}$ (ml STPD/min)	$\dot{V}_E$ (l BTPS) / $\dot{V}_{O_2}$ (l STPD)	RQ	Mechanical efficiency (per cent)
1	L 300	134	32	30 6	955	876	34 9	0 98	20 5
	S 300	146	40	38 5	964	1,070	36 0	0 95	16 0
2	L 400	145	25	23 0	920	900	25 6	0 95	27 3
	S 400	158	27	23 6	875	869	27 2	0 94	28 6
3	L 600	120	20	32 4	1 620	1,414	22 9	0 95	24 7
	S 600	128	19	32 5	1 708	1 547	21 0	0 86	22 2
4	L 500	149	43	52 0	1,209	1 234	42 1	0 95	22 7
	S 500	158	38	49 2	1 295	1 203	40 9	0 95	23 4
5	L 500	174	40	60 3	1 508	1 240	48 7	1 23	23 2
	S 500	174	32	52 8	1 649	1 396	37 8	1 02	20 3
6	L 400	160	24	40 7	1,694	1 097	37 0	1 04	21 1
	S 400	164	20	40 2	2 010	1 084	37 0	1 02	21 4
7	L 200	142	32	32 8	1,023	750	43 7	0 88	17 0
	S 200	151	26	31 5	1 212	836	37 7	0 79	15 0
8	L 500	154	22	44 2	2 008	1 282	34 4	1 01	22 0
	S 500	165	24	44 3	1 848	1 249	35 6	0 97	23 0
9	L 600	145	32	53 4	1 670	1 340	39 9	0 99	26 3
	S 600	154	28	49 7	1 770	1 363	36 4	0 91	25 8
10	S 600	155	58	63 0	1 090	1 507	41 8	0 97	22 2
	L 600	140	47	60 3	1 060	1 489	40 5	1 03	22 6
11	L 400	138	25	28 4	1 134	1 052	27 0	0 87	22 7
	S 400	151	23	27 0	1 174	1 127	24 0	0 82	20 8
12	L 900	153	31	74 1	2 390	2 279	32 5	1 02	21 5
	S 900	164	23	69 5	3 020	2 252	30 9	0 96	21 8

found in case 9 (7 units), but there was only a moderate increase in W_{170} in this case In cases 2, 4, 5, 7 11, D_{LCO} was higher when sitting and there were only moderate increases of W_{170} when in supine

D_{LCO}, sitting, was low (between 1—2 SD) in relation to W_{170} sitting, in four patients (7, 11, 3 and 10) and in one more (case 4) when related to W_{170}, supine In case 9 D_{LCO} was high ($> + 2$ SD)

Discussion

All patients in this study had a clinical picture, chest X ray and biopsy findings indicative of sarcoidosis of the lungs with bilateral hilar lymphomas and also in 3 patiens, with pulmonary infiltrates The patients can from the clinical point of view be regarded as comparable to those earlier reported by Holmgren and Svanborg (16)

The static lung volumes were normal, except in case 7 who almost ranked

with sarcoidosis of the lungs Symbols are those suggested by Pappenheimer et al (27)

V_D (ml BTPS)	P_{aCO_2} (mm Hg)	P_{aO_2} (mm Hg)	$\frac{Q_{SH}}{Q_c} \cdot 100$ (per cent)	Dl_{CO} (ml STPD/min/mm Hg)	pH (units)	Stand bicarb (mE/l)	$P_{IO_2} - P_{AO_2}$ (mm Hg)	$P_{AO_2} - P_{cO_2}$ (mm Hg)	$P_{AO_2} - P_{aO_2}$ (mm Hg)
250	37	89	5 2	21	7 42	23	38	34	22
248	35	93		21	7 45	23	36	43	19
348	42	77	3 6	19	7 36	22	43	38	24
140	39	81		24	7 35	21	41	30	23
219	44	88	5 2	39	7 39	24	45	29	15
251	44	87		33	7 40	25	50	38	12
297	29	—	4 1	28	7 43	20	30	36	—
271	28	110		31	7 44	20	28	32	9
290	31	112	3 6	27	7 38	18	26	37	10
249	31	101		30	7 41	20	30	38	17
74	27	109	4 3	34	7 40	18	25	26	15
110	27	108		31	7 41	18	26	29	11
465	36	63	4 1	14	7 45	24	39	44	45
502	34	69		17	7 45	22	41	40	38
359	33	93	4 1	28	7 45	22	32	37	22
308	30	101		30	7 46	22	30	34	16
476	32	93	6 1	48	7 41	20	32	23	21
520	33	90		41	7 44	20	34	27	22
405	35	94	8 7	29	7 43	21	35	44	20
333	35	92		25	7 40	21	33	48	24
279	40	76	9 7	21	7 40	22	44	41	25
290	42	88		24	7 38	22	48	38	29
301	34	98	5 0	43	7 34	19	37	44	18
645	37	97		43	7 36	19	33	42	15

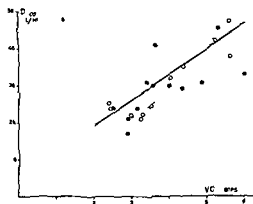
as a group III sarcoidosis i e with rather marked parenchymal infiltrations and signs of fibrosis on X ray Gas mixing was normal in all patients which accords with the results of Holmgren and Svanborg (16)

Analyses of arterial gas tensions showed that alveolar hyperventilation was common during exercise in both body positions, as was also found by Holmgren and Svanborg The difference in heart rate during exercise in the sitting and supine positions was slightly lower in the present material than in that of Holmgren and Svanborg

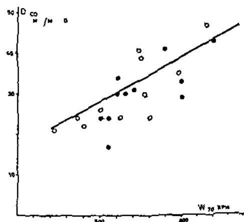
D_{LCO} measured with the steady state technique has been found to vary hyperbolically with oxygen uptake (V_O), (12) in normal subjects The hyperbolic function changes its slope markedly at a V_O that amounts to 40 per cent of the maximum oxygen uptake (12, 20), and measurements above this level of V_O or at a heart rate during exercise above

TABLE III Effect of body pos t on on a number of resp ratory var ables dur ng moderate exerc se in 12 pat ents w th pulmonary sarco dos s analyzed as d fferences between data (s tt ng m nus sup ne) $\bar{x}_L$ — mean value of all (2 n) determ nat ons in sup ne $\bar{d}$ — mean of d fferences $d-(OBS_{sitting} - OBS_{supine})$

Parameter	$\bar{x}_L$	$\bar{d}$	σ d	$\frac{\sigma d \ 100}{\sqrt{2}\ \bar{x}}$	n	t
Heart rate cpm	150 9	9 5	4 2	1 9	12	7 9
Resp rate cpm	30 9	−2 1	4 7	10 8	12	1 5
V_E l BTPS/m n	43 9	−0 9	3 9	6 2	12	0 8
V_T ml BTPS	1 491	119	200	9 5	12	2 1
V_{O_2} ml STPD/m n	1 269	45 8	81	4 5	12	2 0
$\frac{V_E \text{ l BTPS}}{V_{O_2} \text{ l STPD}}$	34 8	−1 9	3 7	7 4	12	1 8
RQ	0 96	−0 1	0 06	4 4	12	3 5
Mech eff per cent	22 2	−0 9	1 8	5 8	12	1 8
V_D ml BTPS	295	9 5	38 7	9 3	11	0 8
$P_{a_{CO_2}}$ mm Hg	34 8	−0 4	1 9	3 8	12	0 8
$P_{a_{O_2}}$ mm Hg	90 9	1 9	6 2	5 0	11	1 0
$D_{L_{CO}}$ ml STPD/m n/mm Hg	29 2	0 6	3 9	9 6	12	0 5
pH un ts	7 40	0 013	0 016	0 18	12	2 8
St b carb mE/l	21 0	0 0	—		12	—
$P_{I_{O_2}}$ $P_{A_{O_2}}$ mm Hg	35 7	0 6	3 0	5 9	12	0 7
$P_{A_{O_2}}$ $P_{c_{O_2}}$ mm Hg	36 3	0 17	5 4	10 5	12	0 1
$P_{A_{O_2}}$ $P_{a_{O}}$ mm Hg	21 8	1 7	4 2	13 7	11	1 3



F g 2 $D_{L_{CO}}$ ml STPD m n mm Hg during moderate exercise in s tt ng pos t on (ord na es) n relat on to v tal capac ty VC l BTPS F lled symbols represent data from the present investigat on open symbols data from a s m lar mater al that of Holmgren and Svanborg (16) Full l ne represents l near regress on of $D_{L_{CO}}$ on VC (12 17 18) $D_{L_{CO}}$ = 7 13 VC + 5 4 SD ± 4 6 n = 38



F g 3 $D_{L_{CO}}$ ml m n mm Hg during moder ate exerc se in s tt ng pos t on (ord nates) n relat on to the rate of work the pa ents could perform at a heart rate of 170 beats m n n s ng pos t on W , kpm m n Symbols as n f g 2 Full l ne represents l near regress on of $D_{L_{CO}}$ on W , in the normal subjects (12 17 18) W , kpm/m n $D_{L_{CO}}$ − 0 0 21 W , + 17 2 SD = 4 6 n 38

TABLE IV Respiratory data including steady state $D_{L_{CO}}$ during exercise at varying loads performed in a varying order (17 18) sitting and supine in three healthy young women

Case	Load (kpm/min)	Heart rate (beats/min)		Resp rate (breaths/min)		$\dot{V}_I$ (l BTPS/min)		$\dot{V}O_2$ (ml STPD/min)		$D_{L_{CO}}$ (ml STPD/min/mm Hg)		V_D (ml BTPS)	
		Sitting	Supine	Sitting	Supine	Sitting	Supine	Sitting	Supine	Sitting	Supine	Sitting	Supine
D A	200	116	113	19	23	19.4	21.1	810	719	25	21	296	315
	400	132	135	20	29	29.4	33.9	1 095	1 050	27	27	342	412
	600	174	168	27	30	38.9	47.2	1 371	1 412	26	27	341	392
I N	400	156	113	25	22	28.5	27.9	889	1 007	32	26	293	245
	600	177	158	30	27	43.0	44.4	1 390	1 417	32	31	352	358
	700		189		32		68.7		1 784		31		558
	800	187		33	[illegible]	49.1		1 723		34		348	
G I	300	114	98	26	18	20.6	20.9	876	998	25	31	318	379
	500	158	155	41	23	30.1	34.4	1 304	1 310	31	35	278	383
	600	138	145	19	18	28.5	28.0	1 443	1 153	37	26	287	330
	800	179	180	24	26	41.5	54.5	1 891	1 875	34	33	244	452

120 beats/min were supposed to give an estimate of the maximum $D_{L_{CO}}$ of the subject. The present measurements were made at an average heart rate of 151 beats/min.

To evaluate the degree of normality of the observed values for $D_{L_{CO}}$ they were compared with those observed in healthy, young trained and untrained men and women (12) figs 2 and 3.

A low $D_{L_{CO}}$ during exercise in the sitting position was then present in four cases when related to W_{170} and VC. The most marked negative deviation from the regression line was found for case 7 (sarcoidosis with parenchymal infiltrations and slight fibrosis) which is compatible with earlier observations (33 34). For the purpose of comparison, the data observed in an earlier material of pulmonary sarcoidosis (group I bilateral hilar lymphomas (16)) have been put together with these figures and the same incidence of low $D_{L_{CO}}$ (i.e. below — SD) has been observed in this material.

The effect of shifting body position on both hemodynamic and respiratory parameters was only slight during exercise in the present material. Apart from the increase in heart rate and RQ there were no significant changes. $D_{L_{CO}}$ did thus not vary significantly with body position. The coefficient of variation for the differences between $D_{L_{CO}}$ supine and sitting was 9.6 per cent, i.e. only slightly higher then that reported for duplicate determinations in the sitting position (17). It is reasonable to assume that the biological variation in a single determination of $D_{L_{CO}}$ should be higher with the present experimental design

TABLE III Effect of body posit on on a number of resp ratory variables during moderate exercise n 12 pat ents w th pulmonary sarcoidosis analyzed as d fferences between data (s tt ng m nus sup ne) $\bar{x}_L$ mean value of all (2 n) determ nat ons in sup ne $\bar{d}$ — mean of d fferences d — (OBS ng—OBS$_{supine}$)

Parameter	$\bar{x}_L$	$\bar{d}$	σ d	$\frac{\sigma d \; 100}{\sqrt{2}\; x}$	n	t
Heart rate cpm	150 9	9 5	4 2	1 9	12	7 9
Resp rate cpm	30 9	−2 1	4 7	10 8	12	1 5
$\dot{V}_E$ l BTPS/min	43 9	−0 9	3 9	6 2	12	0 8
V_T ml BTPS	1 491	119	200	9 5	12	2 1
$\dot{V}_{O_2}$ ml STPD/m n	1 269	45 8	81	4 5	12	2 0
$\frac{\dot{V}_E \text{ l BTPS}}{\dot{V}_{O_2} \text{ l STPD}}$	34 8	−1 9	3 7	7 4	12	1 8
RQ	0 96	−0 1	0 06	4 4	12	3 5
Mech eff per cent	22 2	−0 9	1 8	5 8	12	1 8
V_D ml BTPS	295	9 5	38 7	9 3	11	0 8
P_{aCO_2} mm Hg	34 8	−0 4	1 9	3 8	12	0 8
P_{aO_2} mm Hg	90 9	1 9	6 2	5 0	11	1 0
D_{LCO} ml STPD/m n/mm Hg	29 2	0 6	3 9	9 6	12	0 5
pH un ts	7 40	0 013	0 016	0 18	12	2 8
St bicarb mE/l	21 0	0 0	—	—	12	
P_{IO_2} P_{AO_2} mm Hg	35 7	0 6	3 0	5 9	12	0 7
P_{AO_2} $P_{\bar{c}O_2}$ mm Hg	36 3	0 17	5 4	10 5	12	0 1
P_{AO_2} P_{aO_2} mm Hg	21 8	−1 7	4 2	13 7	11	1 3

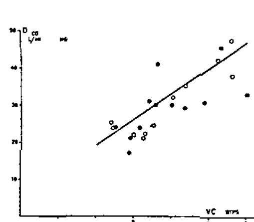
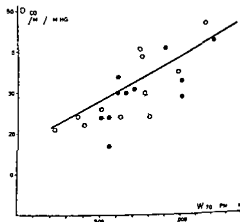


Fig 2 D_{LCO} ml STPD m n^{-1} mm Hg during moderate exercise in s tt ng pos t on (ord nates) in relat on to vital capacity VC l BTPS F lled symbols represent data from the present invest gat on open symbols data from a s milar material that of Holmgren and Svanborg (16) Full l ne represents l near regress on of D_{LCO} on VC, (12 17 18) D_{LCO} 7 13 VC + 5 4 SD ± 4 6 n = 38



F g 3 D_{LCO} ml m n^{-1} mm Hg^{-1} during moderate exerc se n s tt ng pos t on (ord nates) in relat on to the rate of work the pat ents could perform at a heart rate of 170 beats/m n in s tt ng pos t on, W_{170} kpm m n^{-1} Symbols as in fig 2 Full l ne represents l near regress on of D_{LCO} on W_{170} in the normal subjects (12 17 18) W_{170} kpm/min D_{LCO} = 0 0?21 W_{170} + 17 2 SD = 4 6 n = 38

explain the low $D_{L_{CO}}$ demonstrated in the patients who had a low $D_{L_{CO}}$ in relation to pulmonary dimensions but normal lung parenchyma on X ray in the present material or in that reported earlier (16)

Summary

12 patients, 10 women and 2 men, with sarcoidosis of the lungs only bilateral hilar lymphomas in 9 patients, and parenchymal infiltrations in 3 patients were studied with regard to whether $D_{L_{CO}}$ during exercise in the sitting position was lower than in the supine position

$D_{L_{CO}}$ was measured with the steady-state technique during moderate exercise in both sitting and supine positions

A low exercise $D_{L_{CO}}$ in relation to vital capacity and exercise tolerance was found for the sitting position in 4/12 patients

When studied at the same load in supine and sitting body positions, no significant difference in $D_{L_{CO}}$ was observed The variation of the differences between the measurements in sitting and supine positions was of the order of 10 per cent, and within this range changes may have occurred that cannot be detected with the present technique

For the purpose of comparison, the results from three healthy girls studied at different loads when sitting and supine are reported

Acknowledgement

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Hereditary Elliptocytosis and Hyperhaemolysis

A Comparative Study of 6 Families with 145 Patients

By

R A Geerdink, P W Helleman and M C Verloop

The occurrence of numerous elliptical erythrocytes in the peripheral blood (elliptocytosis) was first described by Dresbach (9) The hereditary nature of the abnormality was demonstrated by Hunter and Adams (16 17) and by Hijmans van den Bergh and Rehorst (15) on the basis of a study of a Dutch-American family The pattern of inheritance is that of a simple autosomal mendelian dominant (5) A total of some 800 cases of elliptocytosis (hereditary elliptocytosis) are mentioned in the literature The anomaly is encountered in virtually all ethnic groups with a relatively low frequency For a comprehensive review of the literature we refer to Geerdink (11)

Clinical symptoms which may occur in patients with elliptocytosis can often be traced back to hyperhaemolysis Lambrecht (21) presented the following classification according to the degree of haemolysis in elliptocytosis

A The latent form, in which no signs of hyperhaemolysis are observed

B The compensated form, in which the hyperhaemolysis is compensated entirely by an increased red cell production

C The non compensated form which is characterized by haemolytic anaemia

In the course of a study of the literature by Penfold and Lipscomb (28), the then available methods of determining haemolysis disclosed that about 12 % of cases showed the forms mentioned under B and C In this respect Dacie (8) remarked that this percentage may well have been larger if the mean survival time of the erythrocytes had been determined No systematic investigation into the degree of haemolysis in elliptocytosis has so far been made

Goodall et al (13) were the first to demonstrate a family showing convincing evidence of a close genetic linkage between elliptocytosis and the system o rhesus blood groups These and other linkage investigations were re evaluated by Morton (25), who pointed out that hereditary elliptocytosis may be divided

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genetically into at least two types, depending on the presence or absence of close linkage with the rhesus locus Genetic linkage of properties implies that the corresponding genes are localized close together on the same chromosome

We had occasion to make a study of six Dutch families among which were altogether 145 patients with elliptocytosis One of these families (the De J family) was the Dutch American family previously described by Hunter and Adams (16, 17) and Hijmans van den Bergh and Rehorst (15) In this family, 59 new cases were detected, and a close genetic linkage between elliptocytosis and rhesus factors was found in this family ($P < 10^{-7}$) This linkage could be excluded in three of the remaining families The genetic data were published elsewhere (11, 12)

This paper describes the results of a haematological study carried out on the basis of the following problem definition

a To what extent does elliptocytosis give rise to hyperhaemolysis?

b Is it possible to correlate the above mentioned genetic differences with haematological differences?

Methods

In order to make the diagnosis of elliptocytosis the erythrocytes in a smear stained according to May Grunwald were arranged according to eccentricity ε and divided into the groups suggested by Gunther (14)

$\varepsilon = \sqrt{\frac{1-b^2}{a^2}}$, in which a represents the long axis and b the short axis

Group I round, $\varepsilon < 0.47$

Group II nearly round $\varepsilon = 0.47$—0.62

Group III broad elliptical, $\varepsilon = 0.62$—0.74

Group IV narrow elliptical $\varepsilon > 0.74$

In normal subjects, the number of erythrocytes to be included in groups III and IV together amounts to less than 12 % (14, 27 21)

The literature shows no agreement about the diagnostic criteria of elliptocytosis Lambrecht (21) and Leitner (22) maintained that at least 25 % of the erythrocytes should belong to Gunther's groups III and IV Florman and Wintrobe (10) demanded that at least 40 % of the red cells should be elliptical or at least 10 % of the red cells should be rod shaped Goodall et al (13) considered 33 % elliptocytosis the minimum diagnostic requirement Bannerman and Renwick (2) accepted 25 % elliptocytosis as the requirement for diagnosing hereditary elliptocytosis

In every smear we evaluated at least 200 red cells (from the same microscopic field)

The total serum bilirubin concentration was determined according to Jendrassick and Cleghorn (20) as modified by Schulte (30) The 10 minute percentage as a measure of the conjugated bilirubin level was determined according to Witmans et al (33) The apparent half survival time of the erythrocytes (^{51}Cr T^{1}) was determined with the aid of $Na_2{}^{51}CrO_4$ (32) The serum free haptoglobin concentration, expressed as haemoglobin binding capacity of the serum, was determined by the electrophoretic method according to Nyman (26) At electrophoresis we always added sufficient haemoglobin to permit of a division of the haemoglobin binding capacity of the serum into the classes 0—30 30—60 60—120 and 120—240 mg/100 ml Whenever the haemoglobin binding capacity of the serum proved to be less than 30 mg/100 ml a second electrophoresis was carried out for further division of the haemoglobin binding capacity into the classes 0—5, 5—10 10—20 and 20—30 mg/100 ml

For a description of the other methods used we refer to Dacie and Lewis (7)

Results

In the six families studied, a total of some 400 individuals were examined for elliptocytosis. In 138 blood smears the number of erythrocytes classifiable in Gunther's groups III and IV together was larger than 40 % (usually much larger). All criteria mentioned in the literature (see above) indicate that the diagnosis of elliptocytosis is virtually certain with such a percentage.

In six specimens the number of erythrocytes classifiable in Gunther's groups III and IV together amounted to 30—40 %. The family study showed that such a percentage of elliptical red cells, too, can be a phenotypical characteristic of a gene for elliptocytosis: three of the six individuals examined had children with elliptocytosis. In one specimen the number of red cells belonging to groups III and IV together amounted to 26 %. In our opinion the diagnosis of elliptocytosis is a certainty in this patient too, because two of his children show elliptocytosis. This patient's wife showed a normal red cell picture.

In the smears obtained from the remaining individuals examined (some 250) erythrocytes belonging to groups III and IV together amounted to less than 12 %; this is considered normal (see above).

It is an established fact that increased eccentricity of the red cells can occur in several haemopathies (34). Although in this field work it was impossible to submit all individuals to a comprehensive haematological examination, we believe we can conclude from our findings that in these families there is little risk of secondary elliptocytosis being erroneously evaluated as hereditary elliptocytosis. In a total of 145 individuals in whom the erythrocytes of groups III and IV exceeded 25 %, we diagnosed hereditary elliptocytosis.

The various families showed the following distribution of elliptocytosis: De M. family 32 cases, Van B. family 27 cases, Ri. family 6 cases, Mo. family 12 cases, Van Ac. family 7 cases, De J. family 61 cases. Exhaustive haematological and clinical data on these patients were presented by Geerdink (11).

I. Study of the degree of haemolysis

a. *The serum bilirubin concentration*

The serum bilirubin concentration was determined in 65 patients. The majority of determinations were carried out in the De M. and De J. families. As will be shown later, the bilirubin values in the group of patients in the De M. family were significantly higher than those in the group of patients in the De J. family (cf. fig. 2). The values in the latter family did not distinctly deviate from the normal. In the remaining four families, the serum bilirubin concentration was determined in only a small number of patients. The values we obtained were in our opinion not distinctly different from normal. Consequently it would not be meaningful to compare the bilirubin values of the total group of patients with normal values.

b. *The serum free haptoglobin concentration* (haemoglobin binding capacity)

In 124 patients and 183 normal subjects from the various families the serum free haptoglobin concentration (Hp con-

TABLE I The haptoglobin concentration (haemoglobin combining capacity) in the serum of patients from 6 families with elliptocytosis compared with that of normals from the same families

Free Hp in mg/100 ml	0–30	30–60	60–120	120–240
Elliptocytosis (124 patients)	83	32	9	0
Normal (183 cases)	6	18	104	55

Free Hp in mg/100 ml	<5	>5
Elliptocytosis (124 patients)	61	63
Normal (183 cases)	2	181

TABLE II Haptoglobin concentration in the serum of patients and normals in 6 families with elliptocytosis

Hp content of serum (mg/100 ml)		Total no of cases	0–30	30–60	60–120	120–240
			No of cases			
De J fam (linkage to *rhesus locus*)	Elliptocytosis	52	29	19	4	0
	Normal	73	2	7	43	21
De M fam (no linkage to rhesus locus)	Elliptocytosis	30	28	2	0	0
	Normal	44	2	4	25	13
Van B fam (no linkage to rhesus locus)	Elliptocytosis	21	15	4	2	0
	Normal	42	1	4	23	14
Mo fam (linkage?)	*Elliptocytosis*	11	7	3	1	0
	Normal	13	0	1	8	4
Van Ac fam (linkage?)	Elliptocytosis	7	3	4	0	0
	Normal	8	1	2	3	2
Ri fam (no linkage to rhesus locus)	Elliptocytosis	3	1	0	2	0
	Normal	3	0	0	2	1

centration) was determined The age distribution of the normal individuals corresponded with that of the patients

Table I shows that the Hp concentration in the group of patients was greatly decreased as compared with that in the normal group In all families studied, the patients showed a diminished serum Hp concentration (cf table II) The decreased serum Hp concentration in the total group of patients suggests that hyperhaemolysis exists *in elliptocytosis*

TABLE III Haptoglobin type in 11 patients with elliptocytosis and free haptoglobin in the serum

No of pedigree (see ref 11)		Age in years	Hp-content (mg/100 ml serum)	Hp-type
De M fam				
II	5	82	10–20	2–2
III	18	56	30–60	2–1
IV	39	18	<30	2–1
Van B fam				
IV	16	10	10–20	2–1
IV	25	10	60–120	1–1
Van Ac fam				
III	2	14	30–60	2–1
De J fam				
III	24	77	60–120	1–1
IV	11	69	30–60	2–1
IV	19	55	60–120	1–1
IV	43	46	30–60	1–1
V	37	23	60–120	2–1

In only a small number of patients in whose serum free Hp was demonstrable, was the Hp type determined according to Smithies (31) (cf table III) The number of data available is too small to permit of conclusions This distribution of the Hp types in the families is unknown to us It is a remarkable fact, however, that patients in whose serum free Hp was demonstrable often showed types Hp 2—1 and Hp 1—1 Patients with type Hp 1—1 showed the highest serum Hp values

Individuals with type Hp 1—1 are known to have a greater haemoglobin binding capacity of the serum than individuals with other Hp types (24, 26)

We presume that in some patients with elliptocytosis with type Hp 1—1 only a "relative diminution" of the serum Hp concentration occurs (for instance in one patient with a serum Hp content of 60—120 mg % in three successive determinations the ^{51}Cr T^{1} of the erythrocytes was 20 days)

c *Serum free haemoglobin concentration*

The serum free haemoglobin (Hb) concentration was determined according to Crosby and Furth (6) in 20 patients whose serum contained no demonstrable free Hp and no Hb Hp complex The peroxidase activity was determined with the aid of a Zeiss spectrophotometer PMQ II at 515 nanometer The results are presented in table IV

Determination of the serum free Hb concentration is possible only if the serum contains no Hb Hp complex, for Hb Hp complex has a high peroxidase activity (18 19) and thus can greatly disturb the results of free Hb determinations In normal subjects no free Hb

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	Normal	73	2	7	43	21
De M fam (no linkage to rhesus locus)	Elliptocytosis	30	28	2	0	0
	Normal	44	2	4	25	13
Van B fam (no linkage to rhesus locus)	Elliptocytosis	21	15	4	2	0
	Normal	42	1	4	23	14
Mo fam (linkage?)	Elliptocytosis	11	7	3	1	0
	Normal	13	0	1	8	4
Van Ac fam (linkage?)	Elliptocytosis	7	3	4	0	0
	Normal	8	1	2	3	2
Ri fam (no linkage to rhesus locus)	Elliptocytosis	3	1	0	2	0
	Normal	3	0	0	2	1

centration) was determined The age distribution of the normal individuals corresponded with that of the patients

Table I shows that the Hp concentration in the group of patients was greatly decreased as compared with that in the normal group In all families studied, the patients showed a diminished serum Hp concentration (cf table II) The decreased serum Hp concentration in the total group of patients suggests that hyperhaemolysis exists in elliptocytosis

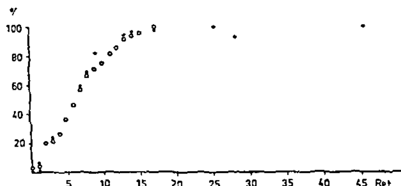


Fig. 1. Cumulative frequency distribution of number of reticulocytes/1 000 RBC.
● = patients } from ell. fam.
○ = normals }
✱ = normal controls

TABLE VI. M:E ratio of the bone marrow in 17 patients with elliptocytosis. Normal ratio (2½–5):1.

	1:1	2:1	3:1	Ratio M:E
De M. fam.	3	3	2	No. of pat.
De J. fam.	–	3	1	No. of pat.
Van B. fam.	–	4	–	No. of pat.
Ri. fam.	–	1	–	No. of pat.
	3	11	3	Total no. of pat.

II. Study of the erythrocyte production

a. *Reticulocyte count*

In 111 patients and 56 normal subjects from the various families and in 49 controls (largely students and laboratory technicians), the reticulocyte count per 1,000 erythrocytes was determined, all determinations being carried out by a single technician. The cumulative frequency distribution of the reticulocyte count per 1,000 erythrocytes is presented in fig. 1.

According to the Smirnov test, there was a statistically significant difference in reticulocyte count between the group of patients and the group of normal controls ($P \sim 2 \times 10^{-5}$). In this respect no difference was established between the normal subjects from the families concerned and the control group ($P \sim 0.20$).

b. *Quantitative ratio between the white and the red cell system in the bone marrow punctate*

This ratio was determined in 17 patients from various families (cf. table VI). In most cases the M:E ratio is slightly diminished. This may be compatible with an increased production of erythrocytes.

TABLE VII Mean values of Hb Hct and RBC in male patients with elliptocytosis (1—6) compared with normal men (7)

Group	Hb in g/100 ml	Hct in %	RBC × 10⁶ /10⁻³ ml	No of pat
1 De M fam	14 0	40 4	4 45	15 ([1]12)
2 Van B fam	14 4	42 1	4 44	11 ([1]9)
3 Ri fam	13 4	42 5	4 24	3 ([1]2)
4 Mo fam	15 6	43 0	4 53	4 ([1]2)
5 Van Ac fam	14 2	42 5	4 46	3
6 De J fam	14 8	43 4	4 50	23 ([1]20)
7 Normals	14 4	41 9	4 40	25 ([1]21)
Standard deviation within the groups	1 45	3 49	0 14	
Critical level (P) by testing with analysis of variance between the groups 1 through 7	0 22	0 78	0 40	
and the groups 1 through 6	0 18	0 93	0 50	

[1] Number of cases above 12 years of age

III Determination of the haemoglobin (Hb) concentration, the haematocrit value (Hct) and the erythrocyte count (RBC) per volume unit of blood

We have attempted to establish whether elliptocytosis exerts an influence on the Hb concentration, Hct value and RBC count per volume unit of blood in 112 patients (59 males and 53 females) and in a control group of 50 normal subjects (25 males and 25 females) from the various families The latter group was so composed as to correspond with the group of patients in age distribution

In the statistical analysis of data, the groups were divided according to sex in view of the small number of data we omitted a division according to age Since the age distribution in the control group corresponds with that in the group of patients, the influence of age in the comparison between patiens and normal subjects is small Graphs in which the Hb, Hct and RBC values have been plotted against the age of the individuals concerned, show that the Hb and Hct values in males under 12 years of age were distinctly below the mean values in the remaining males The data on these boys were not included in the statistical analysis Tables VII and VIII, respectively concerning the groups of male and female patients from the families and the control groups, indicate the mean Hb, Hct and RBC values and their standard deviations within the 7 groups

The same tables mention the tail probabilities P calculated by variance analysis for groups 1 through 7 and groups 1 through 6 No significant differences were established However this does not necessarily mean that no difference exists In order to establish which differences might possibly exist between the true means in the total

TABLE VIII Mean values of Hb Hct and RBC in female patients with elliptocytosis (1—6) compared with normal females (7) from the same families

Group	Hb in g/100 ml	Hct in %	RBC $\times 10^{6}$ $/10^{-3}$ ml	No of pat
1 De M fam	12 8	36 7	4 15	11
2 Van B fam	12 3	39 5	4 32	9
3 Ri fam	11 7	37 5	4 33	2
4 Mo fam	13 2	37 3	4 29	8
5 Van Ac fam	12 1	38 5	4 19	4
6 De J fam	12 6	37 9	4 33	19
7 Normals	12 8	39 0	4 30	25
Standard deviation within the groups	1 14	3 36	0 36	
Critical level (P) by testing with analysis of variance between the groups 1 through 7	0 50	0 40	0 85	
and the groups 1 through 6	0 50	0 65	0 86	

TABLE IX Mean values of Hb Hct and RBC in men and confidence interval for Δ

	Hb in g/100 ml	Hct in %	RBC $\times 10^{6}/10^{-3}$ ml
Groups 1—6 (patients)	14 44	42 28	4 46
Group 7 (normals)	14 38	41 90	4 40
Confidence interval for Δ	$-0\ 51<\Delta<0\ 63$	$-1\ 55<\Delta<2\ 29$	$-0\ 11<\Delta<0\ 22$

TABLE X Mean values of Hb Hct and RBC in females and confidence interval for Δ

	Hb in g/100 ml	Hct in %	RBC $\times 10^{6}/10^{-3}$ ml
Groups 1—6 (patients)	12 61	37 90	4 27
Group 7 (normals)	12 84	39 00	4 30
Confidence interval for Δ	$-0\ 78<\Delta<0\ 22$	$-2\ 72<\Delta<0\ 52$	$-0\ 20<\Delta<0\ 15$

groups of male and female patients (μ) and those in the control groups ($\mu 1$) we calculated 95 % confidence intervals for $\mu-\mu 1$ ($\mu-\mu 1 = \Delta$) by means of the Student distribution These findings are presented in tables IX and X In our opinion the differences are negligible

IV Comparative study of the degree of haemolysis in the various families

As has been described elsewhere (11, 12), close genetic linkage between elliptocytosis and rhesus factors has been demonstrated in the De J family No such linkage existed in the De M,

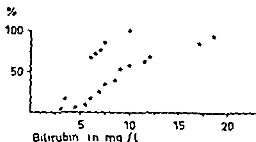


Fig 2 Cumulative frequency distribution of bilirubin content of the serum of the patients in 2 families with elliptocytosis
● = De J fam
✱ = De M fam

Van B and Ri families In the study of the Mo family, only a single report was obtained, on the basis of which linkage must be considered improbable in this family In the Van Ac family, linkage could not be excluded with certainty These findings confirm the data in the literature, indicating the existence of at least two loci for elliptocytosis, one of which is closely linked to the rhesus locus (25)

We also checked whether differences *in degree of haemolysis* were demonstrable between the various families Here we must point out that there was rather a lack of data The only data suitable for statistical analysis were data on the serum bilirubin concentration in the patients of the De M and De J families and data concerning the serum haptoglobin concentration in patients of the De M and De J families

Serum bilirubin concentration

In 22 patients of the De M family and 29 patients of the De J family, the serum bilirubin concentration was determined by the same method Fig 2 indicates the cumulative frequency distribution of the serum bilirubin concentration in the patients of both families

According to the Smirnov test, the De M family differed significantly from the De J family in terms of the patients' serum bilirubin concentrations (P ~ 0 002)

Serum haptoglobin concentration

The free haptoglobin (Hp) concentration of the serum was determined in 124 patients and 183 normal subjects in the various families

Table II summarizes the data on patients and normal subjects per family According to the χ^2 test, a (highly) significant difference was found between the patients of the De M family and those of the De J family (P ~ 0 002), while a (slight) difference was observed between the patients of the De M family and those of the Van B family (P ~ 0 03) In this respect the patients of the De J family did not differ from those of the Van B family (P ~ 0 22)

Discussion

This haematological study was prompted by the problems posed

a To what extent does elliptocytosis give rise to increased haemolysis?

b Is it possible to correlate the genetic differences mentioned in the introduction with haematological differences?

According to the study described in section I (cf pages 717—720 and tables I—V), hyperhaemolysis seems to be the rule in the patients with elliptocytosis examined

The study described in section II (cf page 721, and table VI and fig 1) yielded data compatible with the possibility of increased red cell production in these patients

The study described in section III showed that elliptocytosis does not influence the Hb concentration, Hct value or RBC count per volume unit of blood It seems reasonable to conclude that in our patients with elliptocytosis hyperhaemolysis with adequate compensation by increased erythrocyte production was the rule As we mentioned, Lambrecht (21) used the degree of haemolysis as criterion in dividing elliptocytosis into A a latent form, B a compensated form, C a non compensated form

The elliptocytosis in the families under discussion nearly always came under the B heading It is conceivable that elliptocytosis in general shows the form mentioned under B while the A and C forms constitute exceptions

We have attempted a quantitative approach to the hyperhaemolysis, comparing the degree of haemolysis in the total group of patients with that in normal subjects

It must be pointed out that this quantitative study of the degree of haemolysis is of limited significance because we have found indications of a quantitative difference in haemolysis between the patients of the De M family and those of the De J family (see below) Because of the limited number of data we are unable to eliminate possible quantitative differences in haemolysis between these two families and the remaining four families and among the latter four families In the various families studied, the number of patients differed Comparing the total group of patients from all families together with normal subjects, we run the risk that groups of patients from one or a few families exert a disproportionately strong influence on the totality This quantitative study of haemolysis can give only an impression of the order of magnitude of hyperhaemolysis in elliptocytosis

In some 50 % of our patients we found no haptoglobin or only a trace of it (cf table I)

Investigations made by Brus and Lewis (4) have shown that haptoglobin generally disappears from the serum when the haemoglobin turnover is at least twice as large as normal Relying on these authors, we may conclude that in about 50 % of our patients the haemoglobin turnover must have been at least twice the normal while the mean survival time of the erythrocytes was reduced by (at least) 50 %

The data obtained by determining the mean survival time of the erythrocytes with the aid of $Na_2{}^{51}CrO_4$ are generally in agreement with this conclusion In about half of our 24 cases investigated the ^{51}Cr $T\frac{1}{2}$ value was 21 days or less (cf table V) A decrease in ^{51}Cr $T\frac{1}{2}$ value to 21 days roughly corresponds to a 50 % reduction of the true mean survival time of erythrocytes (29)

The only data suitable for statistical analysis were data on the serum bilirubin concentration and the serum haptoglobin concentration in the patients of the "non linked De M family and the linked De J family

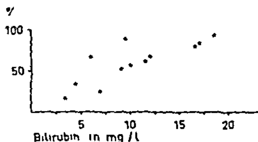


Fig 2 Cumulative frequency distribution of bilirubin content of the serum of the patients in 2 families with elliptocytosis
● = De J fam
✱ = De M fam

Van B and Ri families In the study of the Mo family, only a single report was obtained, on the basis of which linkage must be considered improbable in this family In the Van Ac family, linkage could not be excluded with certainty These findings confirm the data in the literature, indicating the existence of at least two loci for ellip tocytosis, one of which is closely linked to the rhesus locus (25)

We also checked whether differences *in degree of haemolysis* were demonstrable between the various families Here we must point out that there was rather a lack of data The only data suitable for statistical analysis were data on the serum bilirubin concentration in the patients of the De M and De J families and data concerning the serum hapto globin concentration in patients of the De M and De J families

Serum bilirubin concentration

In 22 patients of the De M family and 29 patients of the De J family, the serum bilirubin concentration was determined by the same method Fig 2 indicates the cumulative frequency distribution of the serum bilirubin concentration in the patients of both families

According to the Smirnov test, the De M family differed significantly from the De J family in terms of the patients' serum bilirubin concentrations ($P \sim 0\ 002$)

Serum haptoglobin concentration

The free haptoglobin (Hp) concentration of the serum was determined in 124 patients and 183 normal subjects in the various families

Table II summarizes the data on patients and normal subjects per family According to the χ^2 test, a (highly) significant difference was found between the patients of the De M family and those of the De J family ($P \sim 0\ 002$), while a (slight) difference was observed between the patients of the De M family and those of the Van B family ($P \sim 0\ 03$) In this respect the patients of the De J family did not differ from those of the Van B family ($P \sim 0\ 22$)

Discussion

This haematological study was prompt ed by the problems posed

a To what extent does elliptocytosis give rise to increased haemolysis?

b Is it possible to correlate the genetic differences mentioned in the introduction with haematological dif ferences?

According to the study described in section I (cf pages 717—720, and tables I—V), hyperhaemolysis seems to be the rule in the patients with elliptocytosis examined

Elliptocytosis did not influence the haemoglobin concentration, haemato crit value and erythrocyte count per volume unit of blood The patients therefore showed a compensated hae molysis ,regardless of whether or not they belonged to families in which a genetic linkage between elliptocytosis and rhesus factors exists (i e regardless of whether the corresponding genes are or are not localized close together on the same chromosome)

A comparative study of the degree of haemolysis in the various families disclosed that the patients in one "non-linked family showed a more markedly pathological haemolysis than those in one 'linked' family

This suggests that, not only genetical ly but also biochemically, at least two types of elliptocytosis exist

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Stevens-Johnson Syndrome with Cardiac Involvement

Report of Two Cases

By

STEIN SCHARTUM

Stevens Johnson syndrome is characterized by fever, malaise, affections of the mucous membranes, exanthema and visceral involvement (1) Some authors prefer the term mucocutaneous ocular syndrome (2 8, 9) Cases with predominant skin manifestations are often described as erythema exudativum multiforme Other terms are Behcet s syndrome (2 3, 9, 14) and ectodermosis erosiva pluriorificialis (2, 4, 7, 9) Even Reiters syndrome has been included in this group (9) Much overlapping exists in the symptomatology of these syndromes, but most workers feel that they are just variations of the same disease or mode of reaction in predisposed individuals There may be several aetiological agents although the precise cause, whether viral, bacterial, toxic or otherwise most often fails to be elucidated Non specific hypersensitivity reactions may prove to be responsible for this disease (3, 4 12) The course is variable with a tendency to recurrences A fatal outcome is relatively rare Corticosteroids have been of value in the treatment (3, 7)

The visceral organs are not infrequently involved, especially the respiratory (7, 10) and gastrointestinal tract (2) and the central nervous system (9) Reports of cardiac involvment seem to be few Some hold that clinical evidence of myocarditis may be obtained in Stevens-Johnson syndrome (1, 7), but case reports describing this manifestation are lacking However post mortem findings suggesting myocardial affection have been presented (5 13) Dresner in one case found electrocardiographic changes suggesting transient pericarditis (6) and recently pericarditis has been described in a case of Behcet s syndrome (11) Boe and coworkers on post mortem examination found pericardial effusion in one case of mucocutaneous ocular syndrome (2) Clinical evidence of manifest heart disease in Stevens Johnson syndrome seems to be very uncommon

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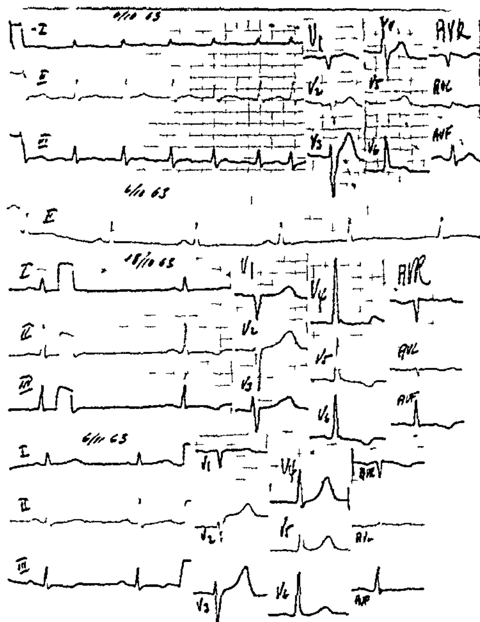


Fig 1 Case 1 Electrocardiograms showing transient atrial fibrillation and pericarditis

Below are presented two cases of this syndrome with atrial fibrillation and in addition in one of the cases pericarditis

Case reports

Case 1 The patient a 49 year old man was admitted to the hospital on October 5 1963 He had previously been healthy Three weeks prior to admission he noted frequency of



Fig 2 Case 1 Roentgenogram showing enlargement of the heart indicating pericardial effusion, and pulmonary congestion



Fig 3 Case 1 Roentgenogram showing normal heart size and lung fields after recovery

micturition and dysuria and was treated with sulphonamides with considerable improvement Two days before admission there appeared an almost generalized rash and he was started on dexamethazone acetate tablets (0 4 mg) of which he took two tablets

On admission his general condition appeared good The temperature was 39 2 C Pulse rate 100/min regular B P 100/70 mm Hg repeatedly unchanged Respirations normal There was a widespread eruption with predelichon for the face backs of the hands and fronts of legs and feet The eyelids were erythematous and swollen Otherwise the lesions consisted of papules vesicles and pustules ranging from a few millimeters to several centimeters in diameter partly confluent On the neck some exudation was noted and on the back brownish red macules There was a moderate conjunctivitis and on the buccal mucous membranes a couple of tiny pustulas The pharyngeal mucosa was inflamed and partly covered with a white exudate No genito-anal involvement was visible Otherwise the clinical examination revealed no abnormalities The urine was positive for protein and in the sediment bacteria and countless leucocytes were seen The haemoglobin content was 82 % Erythrocytes 3 9 mill /mm³ Leucocytes 13 800/mm³ with eosinophilic granulocytes 1 % band formed neutrophilic granulocytes 19 % segmented neutrophilic granulocytes 68 % lymphocytes 8 % and monocytes 4 % Eosinophilic granulocytes 60/mm³ ESR 47 mm/hour Tests for L E cells negative AST 100 Blood culture negative Culture from the skin lesions grew streptococci

On the day after admission the patient was slightly dyspnoeic and subsequent examination revealed atrial fibrillation with a heart rate of 145 beats per minute many of which were frustrated In addition there was a striking pericardial friction rub and at the lung bases moist rales were heard indicating congestive heart failure The electrocardiogram showed atrial fibrillation and elevated S T segments in the standard and precordial leads indicating pericarditis (fig 1) X ray film of the chest revealed enlargement of the heart with a configuration suggestive of pericardial effusion and moderate pulmonary congestion (fig 2) The patient was promptly given digitalis intravenously (Cedilanid 8 ml) and shortly afterwards sinus rhythm reappeared in the electrocardiogram (fig 1)

and the clinical signs of pulmonary congestion were absent after some hours The patient since took digitalis tablets regularly and no relapse of the fibrillation was observed Simultaneously he was treated with hydrocortisone (Actocortin 100 mg) intravenously followed by prednisone 15 mg orally four times a day The friction rub was scarcely audible on the following day while the electrocardiogram showed progressive changes characteristic of pericarditis Transient intra atrial conduction disturbances were noted On discharge from the hospital the electro cardiogram and X ray film were normal (figs 1 and 3)

From the second day after admission there was a striking improvement The tempera ture became normal and the eruption subsided Some of the lesions became confluent and assumed annular and arcuate patterns On the legs the exanthema became petechial and on the backs of the hands there was transient excessive desquamation Very soon however, the lesions crusted and the skin and oral eruptions were completely healed after less than three weeks While the course was otherwise favourable, the patient devel oped a keratitis on the left eye where a corneal ulcer was seen Topical treatment with atropine and antibiotics was applied and the ulcer healed Checking of the urine showed no abnormalities The erythrocyte sedimentation rate fell to normal On dis charge after four weeks except for conjunc tivitis on the left eye the patient had recovered completely Nine months later he was well without symptoms or signs except for slight sequelae of his keratitis

Summary of case report A 49 year old man exhibited fever and lesions of the skin and mucous membranes characteristic of Stevens Johnson syndrome In addition there was evidence of atrial fibrillation and pericarditis with conges tive heart failure There was prompt improvement after digitalis and corti costeroid treatment and complete recovery except for sequelae of keratitis

Case 2 The patient a woman aged 63 was admitted on July 30, 1964 In 1937 a myxofibrosarcoma was removed from her back and postoperative radiation therapy admin istered Otherwise she had enjoyed good health Three weeks prior to admission she noted left chest pain and elevated tempera ture followed by a sparse productive cough She was unsuccessfully treated with penicil lin Terramycin and sulphadiazine

On admission she did not appear seriously ill there was no dyspnoea or cyanosis She was moderately overweight The tempera ture was 39 7 C Pulse rate 102/min regular B P 180/90 mm Hg On the anterior chest and on the back there were areas indicating previous radiation therapy On the feet there were small erythematous eruptions with a few tiny pustulas on the right foot Marked dullness was present at the base of the left lung where breath sounds were distant and rales and pleural friction rub was heard The remainder of the physical examination was within normal limits Laboratory studies revealed traces of protein in the urine and 4 to 5 erythrocytes and about 20 leucocytes in the sediment The haemoglobin content was 72 % Erythrocytes 3 6 mill /mm³ Leucocytes 17,600/mm³ The blood smear showed band formed neutrophilic granulocytes 10 %, segmented neutrophilic granulocytes 64 % lymphocytes 18 % and monocytes 8 % ESR 94 mm/hour Pirquet test positive X ray film showed an infiltration in the left lung In view of the previous unsuccessful treatment she was started on large doses of penicillin and in addition chloramphenicol and streptomycin

After two days of treatment three days after admission an eruption was noted on the back of her left hand There was rubor and swelling forming annular areas and simultanously evidence of conjunctivitis was present on the left eye Further on the same day she exhibited atrial fibrillation the heart rate being 154 beats per minute and the pulse rate 120 There were no signs of congestive heart failure or pericarditis and she had no precordial pain Digitalis treat ment was started and sinus rhythm restored

after some days and permanently established after a brief relapse of the atrial fibrillation The electrocardiogram showed a slightly delayed atrio-ventricular conduction and QRS-changes induced by the digitalis therapy otherwise there were no abnormalities The heart was normal radiographically She was seen by the consultant ophthalmologist and her conjunctivitis was treated with chloramphenicol and boric acid solution After eight days there was complete recovery of the eye affection and healing of the skin eruptions The temperature gradually fell and was normal within ten days after admission The course was so satisfactory that corticosteroid treatment was not considered She had no chest pain while the pleural friction rub remained for a long time No sputum for analyses was obtained Blood cultures and cultures from the skin lesions were negative The urine became normal and the haemoglobin rose to 84 % A mild diabetes mellitus was revealed and controlled by diet restrictions The sedimentation rate fell to 54 mm/hour After four weeks she was discharged from the hospital There was still evidence of left lung infiltration to be controlled in the outpatient department Three months later cells were demonstrated in her expectorate indicating malignancy possibly an adenocarcinoma

Summary of case report A 63 year old woman was admitted with a pneumonia with pleurisy secondary to a malignant tumour in the left lung Before and during the hospital stay she was treated with antibiotics and sulphonamides On the third day after admission skin eruptions and conjunctivitis occurred, compatible with changes in Stevens-Johnson syndrome, and on the same day there was evidence of atrial fibrillation With digitalis treatment sinus rhythm was reestablished after a few days The skin and eye affections healed after two weeks, while there remained signs of an infiltration in the lung after four weeks

Discussion

The cases described above demonstrate that signs of manifest heart disease may be encountered in Stevens Johnson syndrome

Transient atrial fibrillation may be observed in any febrile disease as a non specific phenomenon In case number one however the simultaneous occurrence of pericarditis and fibrillation indicated that organic heart disease was cause of the latter In case number two fever had long been present without accompanying atrial fibrillation which started on the same day as the skin and ocular *manifestations This strongly suggests* that the fibrillation was a result of specific cardiac involvement of the disease

In neither of the patients reported here is the aetiology of the syndrome clear *In case number one the growth* of streptococci from the cutaneous eruptions was a result of secondary infection from a clinical point of view In the same patient initially there were symptoms and signs indicating urinary tract infection, suggesting a bacterial aetiology of the syndrome In case number two although symptoms of urinary tract infection were absent the great number of white blood cells in the urine sediment suggested the presence of an infection *However* such findings are known to occur as an integral part of the syndrome (4) Further, in case number two were an infection the cause of the syndrome, the pneumonia rather than urinary tract infection was responsible Respiratory tract infections are often encountered in Stevens-Johnson syndrome (3 4, 7, 10) whether as a cause

a complication or an integral part of the syndrome has not yet been established There seems to be no relationship between the occurrence of the pneumonia and the severity of the skin and mucous membrane lesions, and there is nothing specific clinically or radiographically about the pneumonia (3) The clinical evidence of pneumonia may antedate the skin eruption (3) In the reported case number two however, the pneumonia was present more than three weeks before the onset of the ocular, cutaneous and cardiac manifestations suggesting it was of aetiological significance In both cases, however, there had been preceding administration of drugs, and there have been many reports of the syndrome following the use of penicillin and notably sulphonamides (3, 4, 5)

In view of the often striking cutaneous exudation that may be seen in this disease it is not surprising to find evidence of effusion in the visceral cavities as well The transient occurrence and the rapid and complete disappearance of the clinical cardiac signs along with the healing of the cutaneous lesions suggest that basically similar pathological changes may have been present Reports of autopsy findings in patients with clinical evidence of cardiac involvement are indeed scanty The loss of striae in the myocardium was demonstrated in one case, without mentioning the clinical course (5) In a case of mucocutaneous ocular syndrome which ran a fatal course signs indicating acute interstitial myocarditis were observed (13)

Whether in the patient reported here the reversion to sinus rhythm together with the swift recovery from the pericarditis can be attributed to the corticosteroid treatment remains uncertain It is felt that the steroid therapy administered effectively prompted the healing

Summary

Clinical evidence of manifest heart disease in Stevens-Johnson syndrome seems to be very uncommon Two cases are reported here demonstrating atrial fibrillation and in one of the cases pericarditis There was complete recovery of the cardiac manifestations

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From the Institute for Cardiovascular Research (Head Jan Brod M D), Prague, Czechoslovakia

A Contribution to the Epidemiology of Chronic Bronchitis

By

V Stanek, J Fodor, Z Hejl, J Widimsky, P Charvát, M Šantruček[1], F Zajíc and M Vavřík

Chronic bronchitis owing to its high morbidity has developed in the last years into a real medical and social problem The pathogenesis is not fully elucidated as yet and further approaches are under consideration One possibility of a new approach to this problem is that of epidemiological studies

Standard methods for the epidemiological research in chronic bronchitis were not developed until the last ten years by English authors (8, 9, 15, 16, 17) According to them, chronic bronchitis is defined as expectoration of mucus on most days of more than three months in at least two consecutive years (7 10) Standardization of the methods and diagnosis make possible various geographical comparative studies Some data of this kind are given (1, 4 13, 23, 24, 27)

The aim of the present study was to reveal the prevalence of chronic bronchitis among men of 60—64 years of age in the district of Prague 2, and to compare our findings with data from other countries

Submitted for publication November 29 1965

Material and methods

An epidemiological study of the male population in the 60 to 64 years age group in the city district of Prague 2 was carried out (12) On the basis of electoral lists it was found that in this district there are living 2 837 men of the given age Out of this total each sixth men was selected a population sample of 473 men being obtained The response was 93 7 % (443 men)

The personal and anamnestic data were recorded in standardized questionnaires In all subjects the following examinations were made Anthropometry venous blood analysis, ECG (12 leads), X ray of the chest in three projections and complete physical examination performed by two trained observers The spirometric examination was performed in the first 99 men The results were evaluated according to the standards of Miller et al (22) Standard criteria were also used for the diagnosis of ischaemic heart disease (31)

The X ray diagnosis of emphysema was made by a single observer on the basis of lung tissue translucency the ratio of peripheral and central vascularisation of the

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TABLE 1 The standard questionnaire

	The whole population sample (443 men) (%)	A Chronic bronchitis present (123 men) (%)	B Chronic bronchitis absent (320 men) (%)	Significance A B
1 Do you usually cough first thing in the morning (on getting up)?	188 (42 4)	119 (96 7)	69 (21 6)	P< 0 0005
2 Do you usually cough during the day or at night?	90 (20 3)	67 (54 4)	23 (7 2)	P< 0 0005
3 Do you cough like this on most days for as much as 3 months each year?	151 (34 0)	110 (90 0)	41 (12 8)	P< 0 0005
4 Do you usually bring up any phlegm from your chest first thing in the morning (on getting up)?	169 (38 1)	117 (95 1)	52 (16 2)	P< 0 0005
5 Do you bring up any phlegm from your chest during the day or at night? (Accept twice or more)	71 (16 0)	64 (52 0)	7 (2 2)	P< 0 0005
6 Do you bring up phlegm like this on most days for as much as three months each year?	127 (28 6)			
7 Do you get short of breath when hurrying on the flat or walking up a slight hill?	248 (56 0)	98 (79 6)	150 (46 9)	P< 0 0005
8 Do you get short of breath walking with others at an ordinary pace on the flat?	119 (26 8)	59 (48 0)	60 (18 7)	P< 0 0005
9 Do you have to stop for breath when walking at your own pace on the flat?	60 (13 5)	38 (30 9)	22 (6 9)	P< 0 0005
10 Are you short of breath on washing or dressing?	61 (13 8)	27 (21 9)	34 (10 6)	P< 0 005
11 In the last three years have you had a chest illness which has kept you off work?	44 (9 9)	24 (19 5)	20 (6 0)	P< 0 0005

lung fields and the position and shape of the diaphragm

The diagnosis of chronic bronchitis was made in each case on the basis of a positive history of mucous expectoration for at least three months of the year Four subjects were eliminated because chronic bronchitis could have been due to left heart failure

TABLE II Clinical and X ray data

	Chronic bronchitis present (123 men) (%)	Chronic bronchitis absent (320 men) (%)	Significance
Family history of pulmonary disease	19 (15 3)	33 (10 3)	n
History of asthma	32 (26 0)	16 (5 0)	P< 0 0005
History of pulmonary tb	18 (14 6)	33 (10 3)	n
Dyspnoea (3rd degree)	38 (30 9)	22 (6 9)	P< 0 0005
Ischaemic heart disease	40 (32 5)	93 (29 0)	n
Blood pressure	149/82	153/85	
Cyanosis	27 (21 9)	23 (7 1)	P< 0 0005
Clubbed fingers	14 (11 3)	13 (4 0)	P< 0 01
Funnel chest	14 (11 3)	13 (4 0)	P< 0 01
Varices	50 (40 6)	94 (29 3)	P< 0 05
Kyphosis of the chest	16 (13 0)	36 (11 2)	n
Scoliosis	9 (7 3)	19 (6 0)	n
Bronchial findings	76 (61 8)	68 (21 2)	P< 0 0005
Plastic pleural alterations +	11 (8 9)	15 (4 7)	n
Plastic pleural alterations + +	3 (2 4)	7 (2 2)	n
X ray signs of emphysema +	19 (15 4)	25 (7 8)	P< 0 05
X ray signs of emphysema + +	9 (7 3)	5 (1 6)	P< 0 01
Peribronchitis	52 (42 2)	89 (27 8)	P< 0 005

TABLE III Vital capacity and forced expiratory volume in 1 sec in per cent of vital capacity 99 men aged 60–64

	Chronic bronchitis present (28 men)	Chronic bronchitis absent (71 men)	Significance
VC (as percentage of predicted)	74 8 %	90 4 %	P< 0 001
FEV %	61 1 %	68 2 %	P< 0 02

Results

Chronic bronchitis was found in 123 men (27 6 per cent) There is a good correlation between positive history of persistent expectoration and dyspnoea of the third degree

In the bronchitis group, varices, cyanosis clubbed fingers and funnel chest were more frequently found than in the others these differences were statistically significant

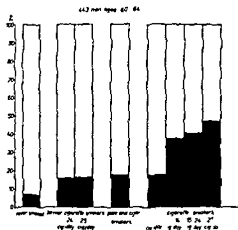
No difference was found between the subjects with and without bronchitis in the anthropometrical data, glycaemia, cholesterolaemia, RBC count, haemo

TABLE 1 The standard questionnaire

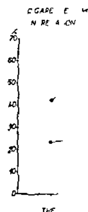
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2 *Do you usually cough during* the day or at night?	90 (20 3)	67 (54 4)	23 (7 2)	P< 0 0005
3 Do you cough like this on most days for as much as 3 months each year?	151 (34 0)	110 (90 0)	41 (12 8)	P< 0 0005
4 Do you usually bring up any phlegm from your chest first thing in the morning (on getting up)?	169 (38 1)	117 (95 1)	52 (16 2)	P< 0 0005
5 *Do you bring up any phlegm* from your chest during the day or at night? (Accept twice or more)	71 (16 0)	64 (52 0)	7 (2 2)	P< 0 0005
6 Do you bring up phlegm like this on most days for as much as three months each year?	127 (28 6)			
7 Do you get short of breath when hurrying on the flat or *walking up a slight hill?*	248 (56 0)	98 (79 6)	150 (46 9)	P< 0 0005
8 Do you get short of breath walking with others at an ordinary pace on the flat?	119 (26 8	59 (48 0)	60 (18 7)	P< 0 0005
9 Do you have to stop for breath when walking at your own pace on the flat?	60 (13 5)	38 (30 9)	22 (6 9)	P< 0 0005
10 Are you short of breath on washing or dressing?	61 (13 8	27 (21 9)	34 (10 6)	P< 0 005
11 In the last three years have you had a chest illness which *has kept you off work?*	44 (9 9	24 19 5)	20 (6 0)	P< 0 0005

lung fields and the position and shape of the diaphragm

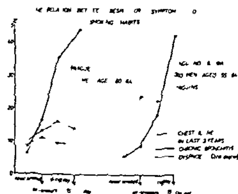
The diagnosis of chronic bronchitis was made in each case on the basis of a positive history of mucous expectoration for at least three months of the year Four subjects were eliminated because chronic bronchitis could have been due to left heart failure



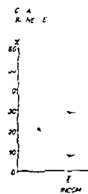
Fg 1 Chron c bronch tis in relat on to smok ng hab ts



F g 3 Gr
II l g t
mod r te ph
phys ac



F g 2 The ncreas ng frequency of chron c bronch tis from non smokers up to men smok ng 15 and more cigarettes a day is of stat st cal s gn ficance in the Prague study



F g 4 In m g ups a f a e income per n nth n l e la rs (rad 1 — m n nal n me gr up l a l V max al income gr up

aetiological factors must be focused only on light smokers and non smokers Due to the small number of non smokers we divided our sample into two groups a) smokers (more than 4 cigarettes a day 222 men) b) light smokers and non smokers (223 men) Fig 3 shows a positive correlation between chronic bronchitis and the degree of physical load Th s positive correlation coul l be explained l y an in reased ratio f sm k ers am ng l eavy labo rers Th s corre lat on was n t estal lished in light smok ers and non smokers l ven the d fferen ces in the frequency of chronic l r n chitis f un l among vari us income gro ips may be explained by the d ffer ent n imber f cigarette sm kers

TABLE V Clinical and X ray data of the investigated groups

	Chronic bronchitis absent (320 men) (%)	Chronic bronchitis chest illness in last three years absent (99 men) (%)	Chest illness in last three years (44 men) (%)	Chronic bronchitis + chest illness in last three years (24 men) (%)
Dyspnoea (3rd degree)	22 (6.9)*+	23 (23.2)∆+	19 (43.1)†	15 (62.5)
Cyanosis	23 (7.2)*+	20 (20.2)+	10 (22.7)*	7 (29.1)
Clubbed fingers	13 (4.1)*+	11 (11.1)+	6 (13.6)*	3 (12.5)
Peribronchitis	89 (27.8)−	40 (40.4)+	15 (34.0)	12 (50.0)
X ray emphysema + signs	25 (7.8)	13 (13.1)	6 (13.6)	6 (25.0)
X ray emphysema + + signs	5 (1.6)*	5 (5.0)	5 (11.3)*	4 (16.6)

Only the first three groups were statistically evaluated. Data marked with the corresponding symbols show statistically significant differences.

There was a negative correlation between the income level (the mean of the income level during the last ten years) and the frequency of respiratory infections, when both extreme income groups are considered. These differences did not depend on differences in cigarette consumption (fig. 4).

Discussion

Our study is limited to the question of the prevalence of chronic bronchitis in a single age group of men, to the problems of its occurrence in relation to smoking habits and to the effect of social factors and work load.

Frequency of chronic expectoration of mucus (27.6 %) is in agreement with data found in population samples with similar cigarette smoking habits in other countries.

Previous research revealed some geographical differences in this respect. Whereas the differences in the frequency of respiratory infections among the individual countries cannot be sufficiently explained, the differences in the occurrence of chronic expectoration may be explained to a high degree with different cigarette smoking habits. All studies, performed until now prove that cigarette smoking is the most important factor in the development of chronic expectoration (5, 6, 21, 28, 30, 32). It is assumed that chronic hypersecretion of bronchial mucus leads to an increased airway resistance, to bronchial obstruction and eventually to emphysema. Some authors demonstrate (14, 20, 33) changes in mechanical ventilation and in lung volumes already in young smokers.

There are possible objections against Fletcher's diagnostic criteria of chronic bronchitis: with this diagnostic approach only smoker's cough is recorded; chronic cough and expectoration in smokers is not always identical with chronic

bronchitis leading to an organic insufficiency, in a number of cases the cough is a question of mere smokers tracheitis

The dramatic change in expectoration which takes place in smokers who stop smoking is well known (3) Our findings show that former cigarette smokers who used to smoke up to 25 cigarettes a day for decades, do not so often suffer from chronic cough and expectoration as do those who smoke less than 5 cigarettes a day Thus persistent expectoration is reversible in most smokers

It may be supposed that smokers with chronic expectoration without a history of respiratory infection should range among these potentially reversible cases of bronchitis We compared subjects with bronchitis without a history of infection with those free of chronic expectoration In men with expectoration of mucus there was a significantly higher frequency of dyspnoea, cyanosis clubbed fingers and X ray signs of so called peribronchitis (table V) We can state therefore, that the expectoration in smokers is in many cases related to an impairment of pulmonary function

Mere history of lung infection in the last three years (without regard to the presence of absence of chronic expectoration) is accompanied by cyanosis clubbed fingers and peribronchitis with equal frequency as Fletcher s diagnosis of chronic bronchitis Dyspnoea and X ray signs of advanced emphysema are even more frequent with past infections Past *lung infections are* obviously related at least in men of this age group to decreased functional pulmonary reserve eventually to pulmonary insufficiency (29) Obviously functional pulmonary reserve may be reduced as a result of manifest pulmonary infections or on the other hand it may be related to chronic bronchial mucus hypersecretion, which has a close correlation with cigarette smoking

What is the relationship between chronic expectoration and respiratory infections? A history of respiratory infection is three times more frequent in subjects with Fletcher s diagnosis of chronic bronchitis as compared with the others At the same time the frequency curves for respiratory infections are not the same as those for chronic expectoration (figs 2 and 4) It is possible that after past infections (which occur among the population independently of chronic expectoration and of excess cigarette smoking (fig 2) hypersecretion of bronchial mucus appears secondarily with predilection for cigarette smokers This is in agreement with the view (15) according to which infection may induce an increased sensitivity to different noxious factors (tobacco smoke dust chemical substances, local meteorological influences) as expressed in hypersecretion of bronchial mucus and eventually in increased disposition to bronchial spasm In some cases however, the disease reaches its terminal stage without history of respiratory infections (26)

Dyspnoea cyanosis and X ray signs of emphysema and peribronchitis are most frequently found in cases with both chronic expectoration and the history of pulmonary infections (table V)

When comparing our findings with data from other countries we find that the frequency of pulmonary infections

TABLE VI The frequency of respiratory syndromes in different studies

Author population	Chronic bronchitis	At least one chest illness in last three years	Cigarette smokers	
Prague 443 men aged 60 to 64 years	27 6 %	9 9 %	Up to 15 cig More than 15 cig a day	19 7 % 33 6 %
Higgins England and Wales 393 men aged 55 to 64 years	25 4 %	22 1 %	Up to 15 cig More than 15 cig a day	44 0 36 1 %
Fletcher London GPO 96 men aged 50 to 59 years	29 2 %	20.9 %	Up to 15 cig More than 15 cig a day	56 2 % 30 2 %
T Mork Bergen Norway 77 men aged 50 to 59 years	11 7 %	15 7 %	Up to 15 cig More than 15 cig a day	0 1 % 16 9 %
Olsen & Gilson Ronne Denmark 183 men aged 55 to 64 years	9 0 %	6 4 %	Up to 15 g tobac More than 15 g tobac a day	44 5 % 25 1 %
Payne Hond & Kjelsberg Tecumsch Michigan 170 men aged 60 to 64 years	13 0 %	12 0 %	Cigarette smokers altogether	47 %

In some studies tobacco consumption is expressed in grams one cigarette is equal to one g tobacco In Denmark there are among the whole male population 38 % pipe smokers and 16 % cigar smokers while only 23 % of adult males are cigarette smokers (cit according to T Mork) Data concerning the sort of cigarettes and tobacco are not available in any study

is highest in England where the course of chronic bronchitis is most severe (2 11 19) *At the same time* the prevalence of chronic bronchitis in England does not exceed that of Prague (table VI) Higher frequency of lung infections among the population does not lead to higher occurrence of chronic expectoration but the course is much more severe in these *conditions*

There remains the question whether our study does not involve two pathogenetically different syndromes leading to an impairment of pulmonary func

tion both of which are usually called in practice chronic bronchitis and emphysema

1 The state characterised by reduction in pulmonary function as a result of pulmonary infection Chronic expectoration may or may not be present dyspnoea being frequent

2 Hypersecretion of bronchial mucus without an infectious component Cough with mucous expectoration is a constant symptom The chief cause of this syndrome is in our opinion, chronic inhalation of cigarette smoke

Both abovementioned states can lead to an impairment of pulmonary function The most serious impairment arises with combination of both Further clinical and epidemiological research must reveal the prognosis of both syndromes and eventually the correction of their differences

Summary

The prevalence of chronic bronchitis was investigated in men aged 60—64, in the city district of Prague 2 A standard questionnaire based on Fletcher s criteria of chronic bronchitis was used

In all subjects a complete clinical examination lung X ray and routine laboratory tests were made Chronic bronchitis was diagnosed in 27 6 per cent of cases The occurrence of chronic bronchitis was significantly correlated with cigarette consumption Increased frequency of chronic bronchitis in the lowest income group and in heavy labourers was also related to higher daily cigarette consumption

The authors discuss the respective roles of pulmonary infections and cigarette smoking in the pathogenesis of chronic bronchitis The findings are compared with those acquired by the same methods in other countries

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From the First Med cal Service Sahlgren s Hosp tal Un ers t G eb g G ebo
Sweden

L-Alpha-Methyl-Dopa Versus the Race — a Comparative Study of the Hypoter

By

KJELL ALESTIG and RUNE SANNER TE

When Gillespie et al (7) in their therapeut c trials with methyldopa in the treatment of arterial hypertension substituted the racemic form (DL AMD) for the L-isomer (L AMD) this was done chiefly because it was found that essentially all the biologic activity of DL AMD resided in L AMD the D isomer being relatively inactive Eventually L AMD became generally known as methyldopa (Aldomet®)

Sjoerdsma in 1961 (13) published the results of alternate doses of DL AMD and L AMD in individual patients finding that both substances had hypotensive effects He states that the transition to L AMD permitted a dose reduction by at least 50 % From the reports of other authors using both forms it is on the other hand not possible to conclude that L AMD gram by gram has a greater hypotensive effect than DL AMD (1 9) No systematic comparative study of the hypotensive effects of the two compounds has however been performed

In some coun but also DL A DL AMD trade n introduced in D after a prelim essentially equ of both compou c aration of two process DL AMD its production is muc been sugge ted that t L perhaps promotes a better a o thereby a hypotens ve effect c m with that of L AMD (8

In view of the Dan sh f ndings it was considered of interest to obtain a systen atic comparison between the hypotensive effects of L AMD and DL AMD with a double bl nd technique

Material

13 in pat ents four males and n ne females — w th ben gn essent al hypertens on were included n the study The average age was 60 years None of the pat ents exh b ted an s gns of cerebral card ac or renal insuff

Subm tted for publ cat on December 22 1965

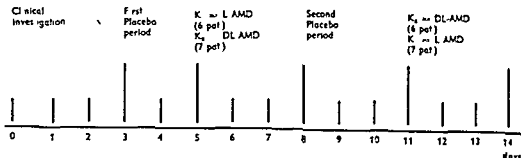


Fig 1 Plan of the experiment

ciency Eight subjects were previously untreated while in three patients previous treatment had been discontinued three weeks or more before the study In two cases previous therapy with chlorothiazide 0 5 g daily and hydralazine 50 mg daily respectively was maintained

Methods

Tablets labelled K_1 (= L-AMD) and K_2 (= DL-AMD) were used together with placebo of identical appearance Each tablet contained 0 25 g of the active substance and the dosage throughout was two tablets three times daily

A plan of the trial is shown in fig 1 Each patient served as his own control In order to avoid confusion with the effect on the blood pressure of the hospitalization per se the first active preparation was not given until the sixth day after admission and in addition the sequence of the active preparations was shifted Each treatment period lasted for three days with a placebo interval of three days in between

In this way two patient groups were created The six patients in group A got L-AMD as the first active drug while DL-AMD was given first in group B of seven patients

Throughout the trial auscultatory blood pressures in the right arm were recorded four times daily in both the lying and standing positions Each recording was preceded by half an hour of rest but apart from this the patients were not confined to bed

The average of all measurements during the last day of each period was calculated for the individual patient and used for compiling the results Differences between the groups were analysed for statistical significance using Student's t test

Results

Group A The individual results are shown in table I Mean values and significant differences are given in table II

From admission to the end of the first placebo period the blood pressure fell from an average of 235/123 to 192/108 mm Hg in the recumbent position L AMD afforded a further reduction in all patients to an average of 145/86 and 136/88 mm Hg in the lying and standing positions respectively, these values being significantly different from those during the first placebo period

During the second placebo period the blood pressure rose to 178 99 lying and 172/102 mm Hg standing but did not reach the level of the first placebo period the differences in standing position being significant From the new and lower placebo level DL-AMD reduced the pressures to an average of 161/90 and 153/95 mm Hg respectively the decrease being significant only f r

TABLE I Group A individual blood pressure values

Blood pressure in mm Hg											
		On admission	Placebo period 1		L-AMD		Placebo period 2		DL-AMD		
Patient	Age	Recumbent	Recumbent	Standing	Recumbent	Standing	Recumbent	Standing	Recumbent	Standing
1	76	270/	224/	211/	131/	110	190	184	1 3	141
		130	120	121	71	70	93	9	83	83
2	54	220/	178/	180/	130/	121/	193	189	159	146
		130	113	125	85	88	118	124	96	104
3	61	260/	169/	181/	121/	118/	149	14	115	115
		125	104	114	83	90	95	9	84	85
4	67	220/	206/	208/	178/	185	196	20	170	185
		110	98	116	88	96	100	105	90	101
5	58	225/	196/	174/	151/	129/	169	158	88	178
		115	99	103	84	81	86	89	39	93
6	43	225/	180/	187/	156/	151/	160	154	8	153
		130	115	117	103	101	104	103	0	106

TABLE II Group A mean values of blood pressure and significant differences

	Blood pressure in mm Hg			
	Recumbent		Standing	
Period	Syst	Diast	Syst	Diast
On admission	235	123		
Placebo period 1	192	108	190	116
L AMD	145	86	136	88
Placebo period 2	178	99	172	102
DL AMD	161	90	153	95
P values for the difference				
Placebo period 1 → L-AMD	< 0 01	< 0 05	< 0 01	< 0 01
Placebo period 1 → Placebo period 2			< 0 05	< 0 05
Placebo period 2 → DL-AMD	–	< 0 05		
DL-AMD → L AMD (B P levels)				

TABLE III Group B individual blood pressure values

Blood pressure in mm Hg

Pat no	Age	On admission	Placebo period 1		DL-AMD		Placebo period 2		L-AMD	
		Recumbent	Recumbent	Standing	Recumbent	Standing	Recumbent	Standing	Recumbent	Standing
1	77	250/120	201/96	199/104	186/81	191/89	201/88	198/93	166/80	171/8[illegible]
2	63	210/125	184/101	196/120	151/93	165/111	153/95	168/116	151/91	149/109
3	51	225/110	160/100	169/113	135/91	149/101	141/91	155/10[illegible]	128/83	131/84
4	71	220/120	204/113	209/124	195/111	205/121	178/104	186/118	175/99	180 10[illegible]
5	48	180/125	145/101	128/105	125/95	114/91	131/95	120/95	118/90	105/91
6	50	220/140	187/118	193/114	175/106	189/119	166/105	183/118	154/100	169 111
7	67	205/110	211/104	203/108	196/93	171/91	205/99	195/101	186/95	1[illegible]9/9[illegible]

TABLE IV Group B mean values of blood pressure and significant differences

Period	Blood pressure in mm Hg			
	Recumbent		Standing	
	Syst	Diast	Syst	Diast
On admission	216	121	—	—
Placebo period 1	185	105	185	113
DL-AMD	166	96	169	103
Placebo period 2	168	97	172	107
L-AMD	154	92	[illegible]	97
P values for the difference				
Placebo period 1 → DL AMD	<0 01	<		05
Placebo period 1 → Placebo period 2	<0 01			
Placebo period 2 → L AMD	<0 05			
DL-AMD → L-AMD (B P levels)	<0			

TABLE V Group A compared with group B mean values of blood pressure reductions on L AMD and DL-AMD respectively and test of significances

Treatment period 1	B P reduction in mm Hg				Treatment period 2	B P reduction in mm Hg			
	Recumbent		Standing			Recumbent		Standing	
	Syst	Diast	Syst	Diast		Syst	Diast	Syst	Diast.
Group A					Group A				
L-AMD	48	23	55	28	DL-AMD	17	9	19	7
Group B					Group B				
DL-AMD	18	9	16	9	L-AMD	14	5	17	9
	$p<0.01$	$p<0.05$	$p<0.01$	$p<0.05$		$p>0.1$	$p>0.1$	$p>0.1$	$p>0.1$

standing diastolic pressure In one case there was no obvious hypotensive effect at all with DL AMD

In respect of the blood pressure levels obtained with the two preparations L AMD achieved the better result, in spite of the fact that it was administered first The difference is, however, not significant

Group B Table III contains the results for the individual patient and table IV mean values and significant differences for the whole group

There was in this group a significant reduction in blood pressure from admission to the end of the first placebo period or from an average of 216/121 to 185/105 mm Hg in the recumbent position During DL-AMD both the systolic and diastolic pressures then decreased significantly in the two positions the figures being from 185/105 to 166/96 and from 185/113 to 169/103 mm Hg respectively

As the second placebo period resulted in only a slight average rise in pressure, the starting level before L-AMD administration was about the same as that obtained with DL-AMD Even so, L-AMD brought about a further reduction in all patients to an average of 154/92 and 155/97 mm Hg in the lying and standing positions respectively These values are significantly lower than those of the second placebo period

When the levels achieved with the two substances are compared, L-AMD exerted a better effect with significantly lower systolic values in both positions

Group A versus Group B As the two patient groups are equally composed, with the same average age and inconsiderable differences in initial blood pressure levels a comparison between the groups has been considered to be justifiable (table V)

During the first treatment period L-AMD produced significantly greater decreases than DL-AMD in all pressures registered, while there are no significant differences between the active preparations during the second treatment period The initial blood pressure levels before the last period were however considerably lower

Side effects

No side effects of any importance were seen A woman 76 years old, complained of dizziness during L-AMD Slight tiredness was noticed in some patients with both substances

Discussion

The present trial was planned as a double blind study, each patient being *his own control The blood pressure level* during the second placebo period was, however, not restored to the initial level in any group Published reports state that alpha methyl dopa is almost completely excreted within 24 hours, provided that renal function is normal When the drug is discontinued the blood pressure will usually reach the starting level within 12—48 hours (2, 5, 7) It may therefore be presumed that the lower level at the end of the second placebo period was mainly the result of continuous hospital rest, even though a persisting hypotensive effect of the first administered drug cannot be excluded Longer placebo periods would have been necessary to clarify this, but were not possible for practical reasons

Ideally there should also have been somewhat longer treatment periods in order to obtain reliably stabilized blood pressure levels The hypotensive effect of alpha methyl dopa will, however, usually appear within 2—24 hours with a maximum after 12—72 hours (3, 4, 5, 11, 12) This together with the relatively large dose of 1 5 g a day should presumably have ensured the detection of any blood pressure reduction during each three day period of treatment

The results show that both substances exert a significant hypotensive effect However, DL-AMD was effective only when given as the first active preparation, whereas L-AMD produced significant blood pressure reductions irrespective of whether or not it was given first Furthermore the pressure levels obtained with L AMD were lower in both groups, with significantly lower systolic pressures in both the recumbent and standing positions in group B

It seems therefore as if L-AMD 1 5 g daily is more effective than DL-AMD in the same dosage as regards the hypotensive effect The study will, however, not permit an exact grading of the hypotensive action of the two substances

The present results contradict the presumption that DL-AMD, by reason of a better solubility in vitro, should be more easily absorbed than L-AMD, thereby reaching an identical hypotensive effect weight for weight The absorption studies available also indicate rather better absorption of *L-AMD than* of DL-AMD (2, 5, 6, 7) In the long term treatment of arterial hypertension larger amounts of DL-AMD than of L-AMD will therefore very likely be needed, if the same *hypotensive response* is aimed at

If DL AMD should prove to be economically more profitable for the patients, in spite of the larger doses necessary, this will be a *factor of importance* Against a routine use of DL-AMD stands, however, the general principle of keeping the amount of foreign substances in the body as low as possible because of the risk of *toxic effects* Since

the therapeutic dose of L AMD already is 1 5 g daily or more in many cases, any increase in the amount administered would moreover, be an inconvenience to the patient

Summary

The short term hypotensive effects of the racemic form and the L isomer of alpha methyl dopa were compared in 13 hospitalized patients with arterial hypertension A double blind technique was used the patients serving as their own controls

After a placebo period the active preparations in a fixed dose of 1 5 g daily were administered for three day periods separated by a second placebo period of three days, the sequence of the active drugs being alternated Auscultatory blood pressures in the recumbent and standing positions were recorded four times daily

Both substances were shown to exert significant hypotensive effects The L isomer produced significant blood pressure reductions irrespective of whether or not it was given first, whereas the racemic form was effective only when given first The blood pressure levels obtained with the L isomer were throughout lower than those with the racemic form

The place of the racemic form in the long term treatment of arterial hypertension is discussed

Acknowledgement

Coded tablets containing 0 25 g of DL alpha methyl dopa and L alpha methyl-dopa respectively and an identical placebo preparation were placed at our disposal by AB Erco Stockholm Sweden

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Antibody Deficiency in Paraproteinemia

By

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Clinical observations of patients suffering from multiple myeloma (M M) and essential or secondary paraproteinemia (5 43, 61) have revealed frequent occurrences of bacterial infections in these diseases Numerous experimental data (7, 18, 35 37, 38, 42, 63) have univocally confirmed that impairment of antibody production is a typical aspect of M M

The phenomenon is generally explained by the secondary hypoimmunoglobulinemia, due to the malignancy of immunoglobulin producing cells Indeed, although the total amount of plasma globulins is often increased, a reduction of normal serum immunoglobulins has been demonstrated (16 23, 36 48) The serum myeloma proteins are referred to as paraproteins' (1) i e abnormal globulins structurally related to the normal γ globulins but in some way antigenically deficient (17, 19 21, 22 33, 34 50) They give rise to characteristic sharp protein peaks in electrophoresis (M component (51)) and a deflecting arc in immuno electrophoresis (5 24)

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Because of a structural abnormality, one could not expect paraproteins to show antibody specificity (3), and Osserman and Takatsuki (45) report several unsuccessful attempts to demonstrate such antibody activity

The present study was undertaken in order to investigate the spontaneous occurrence of antibodies against widespread bacteria, in sera from patients with M M and other forms of para proteinemias, and to ascertain the role of isolated paraproteins in this immuno logical responsiveness

Materials and methods

Sera

Sera from 93 patients with paraproteinemia were examined The patients were hospitalized in Danish hospitals and blood was drawn routinely for the purpose of diagnosing the possible presence of a paraprotein in the serum In the routine screening two to four cases of paraproteinemia per week were found

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TABLE I Number, age and sex distribution of paraproteinemic patients and controls

	No	%	Age		Sex			
			Mean	Range	♂	%	♀	%
γ_A paraproteinemia	27	29	66.4	52–83	18	66.6	9	33.3
γ_G paraproteinemia	66	71	65.3	34–94	38	57.5	28	42.5
Normal controls	40	—	38.6	15–70	22	55.0	18	45.0

in hospitalized individuals from a population of about 2 million

40 sera, obtained from normal persons in apparent good health were used as controls None of the donors had had a febrile illness or other diseases in the last six months

The number of paraproteinemic and normal sera tested, as well as sex and age distribution (median value and range) are summarized in table I

Criteria for the diagnosis of paraproteinemia

In all cases an abnormal, sharp peak or M component (51) was detected by agar gel electrophoresis (65) in different electrophoretic areas from α_2 to the slow γ zone This M component' was identified as γ_G or γ_A globulin (WHO meeting on Nomenclature of human immunoglobulins Prague 29—30 May 1964) by immunoelectrophoresis carried out as described by Grabar (23) modified as a micromethod according to Scheidegger (53) Polyvalent horse (No 2292 from the Central laboratory of the Netherland Red Cross Blood transfusion service Amsterdam) and goat antisera against pooled normal human serum and strictly specific anti γ_G anti γ_A and anti γ_M globulin rabbit antisera were used (5) The protein patterns and precipitates were stained with amido-black (65)

By identification of the paraprotein type the pathological sera were classified into two groups 1 66 cases containing γ_G paraproteins (syn γ 7S γ 6.6S γ γ_2 γ_{ss}) 2 27 cases containing γ_A paraproteins (syn β_{2A} γ_{1A}) Association of both γ_G or γ_A paraprotein and γ_L type paraprotein (Bence Jones protein) was observed in several cases Myeloma sera with no detectable abnormal protein and sera showing only Bence Jones protein were not considered for this study Neither were hypoimmunoglobulinemic sera from patients with lymphatic leukemia and macroglobulinemia investigated (9 56)

Proteins

Total protein content of sera and other samples was determined by Lowry's method as described by Lowry et al (40) Semi-quantitative estimation of the anomalous protein was thereby possible from the relative distributions of the electrophoretic fractions The electrophoreses were scanned at 370 mμ in a Vitatron photometer connected to a scanning device and automatic recorder This wave length gives rise to a minimum of absorption of light, so that even dense protein peaks like those found with paraproteins show extinction values of a size still obeying Beer's Law

Isolation of the anomalous protein was carried out in 21 sera available in larger quantities 16 with γ_G paraproteinemia and the other 5 with γ_A paraproteinemia About 5 ml of serum were diluted with 2 volumes of tap water (conductivity 432 μ Siemens pH 6.5) and a 4.0 M solution of ammonium sulphate (pH 6.8) was then added step by step Most M components were precipitated at 2.0 M ammonium sulphate After dialysis for 16 hours against running distilled water, the precipitates of every step usually at 1.0 1.5 2.0 2.5 3.0 M ammonium sulphate were controlled qualitatively and quantitatively by immuno-electrophoresis and agar

gel-electrophoresis (4) Further purification of the anomalous protein was achieved by column chromatography on DEAE cellulose using an elution system with constant pH ($pH = 8.0$) and molarity continuously changing from 0.02 M (conductivity 1 900 micro-Siemens) to 0.30 M (conductivity 16 000 micro-Siemens) of the phosphate buffer (15). Hereby the paraproteins of the γ_G type were isolated in fractions with low molarity (in general from about 2 000 to 5 000 micro-Siemens of conductivity). The isolated paraproteins were finally concentrated by vacuum dialysis to a concentration corresponding to that calculated in the whole serum.

16 out of 21 isolated paraproteins (15 of the γ_G type and 1 of the γ_A type) were judged sufficiently pure for precipitin reactions according to the following criteria:

1. Presence of a single M-gradient on agar gel-electrophoresis with relative mobility corresponding to that found in the serum.
2. Immuno-electrophoretic evidence of a single deflecting arc with the same characteristics found in the serum. The exclusive presence of one single deflecting arc was always demonstrated using horse and goat antisera to normal human serum as well as strictly specific antisera to γ_G, γ_A and γ_M globulin.

Figs 1 and 2 show the agar gel-electrophoretic and immuno-electrophoretic patterns of a γ_G myeloma serum and of the corresponding isolated paraprotein.

Antigens

Strains of bacteria belonging to staphylococci, streptococci and pneumococci were cultured on solid medium (agar bouillon for staphylococci and agar Difco N I H B 258 for streptococci and pneumococci). Cowan's types 1, 2, 3 and 4 (8, 31) were chosen for *Staphylococcus aureus*; types faecalis, EC 2, EC 5, EC 6, EC 7 and EC 8 for *Streptococcus pyogenus* and types EA 1a, 1b, 2a and 2b (41) for *Diplococcus pneumoniae*.

The cultures obtained from each of these types were scraped off and washed three times with saline, the microorganisms being isolated each time by centrifugation (5 000 g, 10 min). The soluble proteins were extracted from the bacteria by means of an X press homogeneizer (13). Hereby a disruption of the microorganisms was obtained; the suspensions were allowed to stand 24 hours at 4° C after 10 minutes of ultraviolet irradiation and then centrifuged at 12 000 g for 1 hour.

In order to evaluate the protein pattern of the bacterial extract, total protein determination and agar gel-electrophoresis were performed on the clear supernatant. Fig. 3 F shows the pattern of Cowan's type 1 *Staphylococcus aureus*.

It is known that although every strain of staphylococcus possesses an individual mosaic of antigens, at least three common antigenic determinants can be found in all of them (8, 31). One of these is the so called "A antigen". Because of its strong antigenic properties, homologous antibodies against it can be detected in practically every normal serum (31).

An attempt was made to isolate the A antigen. When Cowan's types 1 and 3 extracts were submitted to DEAE chromatography (15), 2 peaks were always obtained corresponding to the fractions possessing from 1 900 to 4,500 and from 7 700 to 9 700 micro-Siemens of conductivity, respectively. The fractions corresponding to each peak were pooled and concentrated 100 times by vacuum dialysis; however, when tested in Ouchterlony plates against normal serum, only the second peak gave rise to a precipitate and yielded an identity reaction with the whole bacterial extract. A rabbit specific antiserum against Cowan's type 1 extract reacted in immuno-electrophoresis specifically with the second peak, but not with the first one (fig. 3 A and B). Agar-gel-electrophoresis of the second peak revealed 2 fractions in the prealbumin albumin and in the α areas. Finally, the whole bacterial extract and the chromatographic second peak were run on the same slide by Wieme's technique: after the electrophoretic separation a trough was cut between the two splits and a positive normal human serum was allowed to diffuse; in this way a faint protracted precipitate with slight double curvature appeared on

TABLE II a Occurrence of precipitating *antibodies in paraproteinemic* and normal sera (Ouchterlony technique)

Antigens[1]			A paraproteinemia (27 cases)		G paraproteinemia (66 cases)		Controls (40 cases)	
			Positive	%	Positive	%	Positive	%
Staphylococci	Cowan 1	(2 9)	24	88 8	61	92 4	40	100
	Cowan 2	(3 1)	1	3 7	2	3 0	5	12 5
	Cowan 3	(2 4)	24	88 8	60	90 9	40	100
	Cowan 4	(2 0)	4	14 8	10	15 1	15	37 5
Streptococci	Faecalis	(6 5)	1	3 7	2	3 0	6	15
	EC 2	(5 7)	3	11 1	7	10 6	10	25
	EC 5	(2 5)	2	7 4	2	3 0	7	17
	EC 6	(5 2)	4	14 8	9	13 6	12	30
	EC 7	(6 2)	7	25 9	13	19 6	15	37 5
	EC 8	(3 7)	2	7 4	3	4 5	11	27.5
Pneumococci	EA1a	(4 5)	5	18 5	11	16 6	23	57 5
	EA1b	(2 7)	1	3 7	3	4 5	13	32.5
	EA2a	(2 2)	2	7 4	7	10 6	15	37 5
	EA2b	(3 0)	2	7 4	3	4 5	10	25

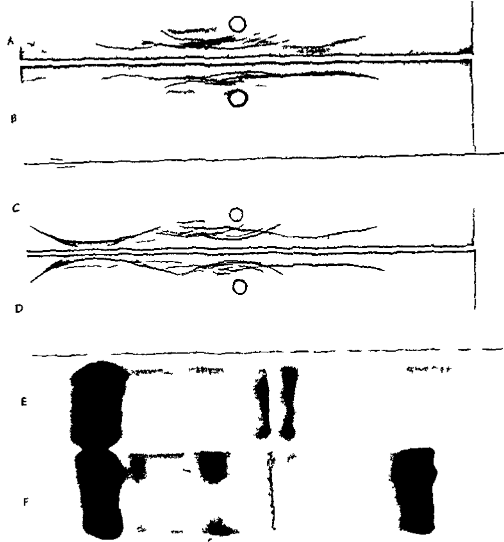
[1] The number in parentheses beside each bacterial strain indicates the protein content in g/100 ml of the extracted soluble antigens

both sides of the trough It was, therefore, *assumed that the second chromatographic* peak corresponded to the A antigen of the bacterial extract Carbohydrate determina*tions gave the following results fucose (54)* 29 μg/mg, hexosamine (2 14) 6 μg/mg, glucuronic acid (10) 9 μg/mg

Immunological tests

Ouchterlony gel double diffusion test (46), as well as *micro-immuno-electrophoresis* were used throughout the work for detecting the occurrence of antibodies against the *bacterial antigens tested* The Ouchterlony diffusion test was performed by means of a LKB microequipment (LKB Ltd Stock*holm Sweden) using 5 μl of each sample* The distance between edges of the central well and peripheral wells was 5 2 mm in the diagonal pattern and 5 0 in the circular pattern, the diameter of each well was 3 0 mm Total protein content of the bacterial *strains, which were of the same batch* throughout the experiments is reported in table II a Undiluted normal and paraproteinemic sera were used and the isolated paraproteins contained from 0 7 to 7 3 g % protein (table IV) A positive antigen*antibody reaction* always gave a visible precipitate and the antigenic interpretation of the findings was based upon the criteria of *Ouchterlony (46)*

A modified precipitin technique for the quantitative estimation of the antibody con*tent was employed* (see the review by Kabat and Mayer (32)) The thermo-labile complement fraction of the serum was removed either by heating it at 56 C for 30 minutes or by allowing it to stand for more than 8 days at 4 C. Sera and antigens were always carefully centrifuged (10 000 g for 20 minutes in order to get a clear supernatant An 8 [illegible]



I g 1 Immuno-electrophores s and agar gel m cro-electrophores s of the serum from a pat ent w th mult ple myeloma Abo e the mn uno-electropl ores s is de eloped v th a goat ant serum aga nst normal pooled human serum Below the immuno electrophores s is developed with a horse antiserum aga nst normal pooled human serum A immuno-electrophores s of the myeloma serum The deflect ng parapro e n prec p tat on arc can be seen B mmuno-electrophoresis of a normal human serum C the same as A D the same as B E agar gel m cro electrophores s of a normal human serum F agar gel m cro-electrophoresis of the same myeloma serum as A and C. An abnormal M componen can be seen

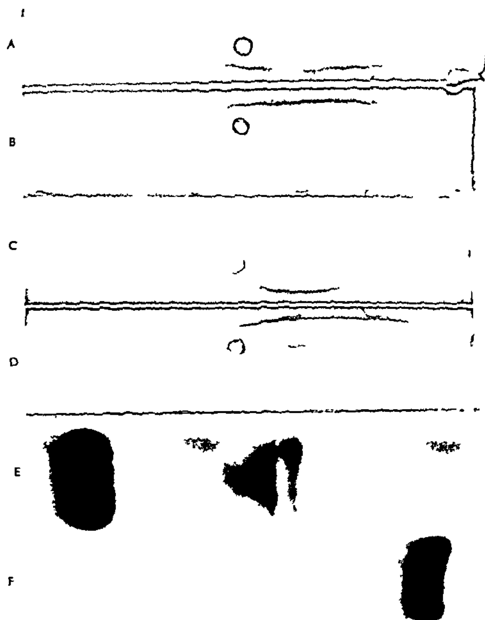


Fig 2 Immuno-electrophoretic and agar gel m[illegible]ro-electrophoretic p[illegible]terns of the paraprotein isolated from the myeloma serum illustrated in Fig 1 Above the [illegible] un[illegible]electrophoresis [illegible] developed with a horse antiserum against normal pooled serum Below the immuno-electrophoresis [illegible] developed with a rabbit antiserum aga[illegible]st normal human [illegible]-globulin A [illegible] u[illegible]electrophoresis of the isolated paraprotein The ex[illegible] presence [illegible] single [illegible] precipitation [illegible] can be seen B [illegible] immuno-electrophoresis [illegible] normal human [illegible]-globulin C the same as A D the same as B E agar gel microelectrophoresis of a normal human serum F agar-gel microelectrophoresis of the isolated paraprotein Only the abnormal M-component is visible

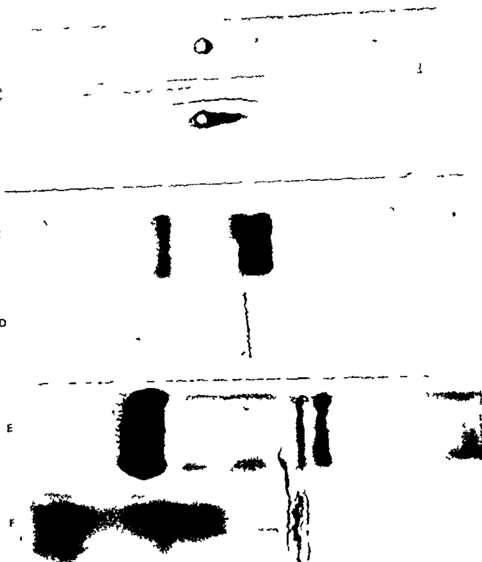


Fig 3 A and P immuno-electrophoresis of the pooled concentrated fractions corresponding respectively to the first and second peak obtained by *DFAF* chromatography of the soluble antigens from Cowan's type 1 staphylococcus aureus Both the pooled fractions are developed with a rabbit specific antiserum against Cowan's type 1 The fractions corresponding to the second peak gave also an immuno-precipitate with sera *precipitating the* "A antigen Thus the second peak corresponds to the A antigen of the staphylococcus extract C agar gel micro-*electrophoresis* of a 3° mixture of immunological pure albumin and transferrin plus dextran as *mobility* standards D agar gel micro-electrophoresis of the isolated A *antigen* E agar gel micro-electrophoresis of a normal human serum F agar gel micro-electrophoresis of the soluble bacterial antigens extracted from Cowan's type 1

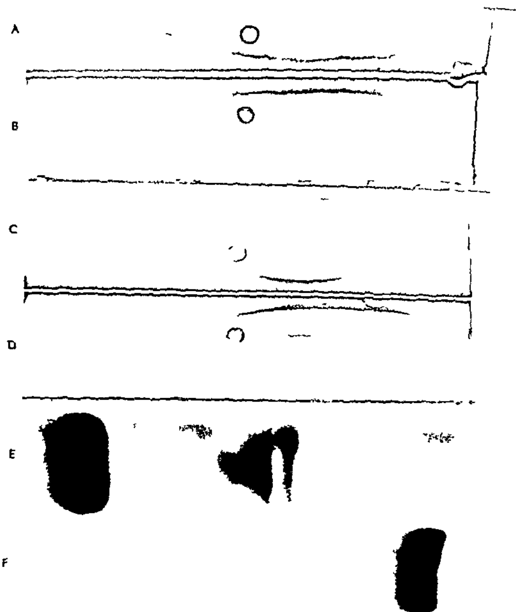


Fig. 2. Immuno-electrophoretic and agar gel micro-electrophoretic patterns of the paraprotein isolated from the myeloma serum illustrated in fig. 1. Above the immuno-electrophoresis is developed with a horse antiserum against normal pooled serum. Below the immuno-electrophoresis is developed with a rabbit antiserum against normal human γ globulin. A: immuno-electrophoresis of the isolated paraprotein. The exclusive presence of one single deflecting precipitation arc can be seen. B: immuno-electrophoresis of a [illegible] solution of normal human γ globulin. C: the same as A. D: the same as B. E: agar gel micro-electrophoresis of a normal human serum. F: agar gel micro-electrophoresis of the isolated paraprotein. Only the abnormal "M component" is visible.

NaCl solution containing 0 05 M phosphate buffer (pH 7 5) was employed for diluting the samples

The amount of antigen to be used was determined by a preliminary experiment in which successive dilutions of antigen were added to 500 μl of a positive pooled normal serum Under these circumstances a concentration of antigen of 600 μg per 100 μl was found to assure a slight excess

Normal human γ_G globulin solutions containing from 2 to 200 μg γ_G globulin per 200 μl were used to determine the standard curve To each tube 500 μl of antigen (diluted according to the preliminary tests) were added and the mixture was allowed to stand for 1 hour at room temperature The precipitate formed was isolated by centrifugation (10 000 g for 15 minutes) and washed three times with the NaCl solution described above After drying in an electric oven (100 C) the immuno-precipitate was treated with 200 μl 0 1 N NaOH and the protein content estimated photometrically by Lowry s method (40)

A standard curve was obtained by plotting the extinction values E_{750} nm as a function of the protein content A rectilinear relationship exists in the interval from about 35 to 140 μg γ_G globulin per 200 μl When a positive pooled normal human serum (total protein content = 7 6 g %) was tested the rectilinear relationship was found to correspond to dilutions of the serum from 16 to 64 times

12 positive paraproteinemic sera and the corresponding isolated paraproteins (11 of the γ_G type and 1 of the $_A$ type) were investigated for their antibody content against staphylococcus extracts and isolated A antigen

Results

Antibodies against streptococci and pneumococci

The spontaneous occurrence of circulating antibodies against the bacterial antigens tested in paraproteinemic sera and normal controls is summarized in table II a

Antibody synthesis against the 6 strains of streptococcus and the 4 strains of pneumococcus appears depressed in paraproteinemic sera under the experimental conditions used, the amount of positive cases, especially against the pneumococcus antigens, are constantly lower than those found in normal controls, and differences are always statistically significant ($p < 0.01$) However, a general correspondence between normal and paraproteinemic sera is still present antibodies which are more frequently encountered in normal sera show the same behaviour in pathological sera, and vice versa

Although not all normal controls possess antibodies against every one of the antigens investigated in these two classes the presence of antibodies against several strains of the same bacterial class is much more common in normal controls The complete lack of all antibodies was found only in 7 5 % of controls, whereas the relative percentage for all paraproteinemic sera is 59 % There fore impairment of antibody production appears even more pronounced if the antibody occurrence against streptococci and pneumococci is considered regardless of the different strains against which the antibodies were detected in these conditions positive tests were obtained in 55 %, of controls and 25 % of paraproteinemic sera and in 75 % of controls and 23 % of paraproteinemic sera using streptococcus and pneumococcus extracts as antigens respectively

It also appears from table II a that no relationship can be established between

TABLE II b Simultaneous occurrence of antibodies against extractable antigens from different bacterial classes (Ouchterlony technique)

	γ_A paraproteinemia (27 cases)		γ_G paraproteinemia (66 cases)		Controls (40 cases)	
	No	%	No	%	No	%
1 Antibodies against staphylococci and streptococci	8	29 6	16	24 2	22	55
2 Antibodies against staphylococci and pneumococci	6	22 2	15	22 7	30	75
3 Antibodies against streptococci and pneumococci	2	7 4	5	7 6	16	40
4 Antibodies against staphylococci streptococci and pneumococci	2	7 4	5	7 6	16	40

deficiency of circulating antibodies and type of anomalous protein decrease in antibody production occurs in the γ_A- as well as in the γ_G-type Sex and age distribution also seems to be of no importance An attempt was made to correlate the electrophoretic pattern *with the immunological responsiveness* Total protein content, amount of anomalous protein and albumin/globulin ratio were considered, however no relationship could be similarly established for any of these parameters

Antibodies against staphylococci

As it appears from table II a, the antibody synthesis is depressed in the two groups of paraproteinemic sera only as far as Cowan's types *2* and 4 antigens are concerned in these cases, in fact, the percentages of positive cases are significantly lower than those calculated for normal sera ($p < 0\ 01$) Conversely, when Cowan's types 1 and 3 were tested small differences *without statistical significance* were noticed between paraproteinemic and normal sera

The striking occurrence (100 %) of antibodies against these two staphylococcus strains in the control group is in agreement with the results obtained by Jensen (31)

*The varying amount of A antigen pres*ent in each strain may account for the different percentages of positive cases found with the four strains investigated in this study

Simultaneous occurrence of several antibodies

The occurrence in the same sera of antibodies against two or more strains of different bacteria is recorded in table II b Here again the antibody synthesis appears depressed in paraproteinemic sera as compared with normal controls, without any apparent relationship to the type of the anomalous protein In particular the coexistence in the same serum of antibodies against streptococci and pneumococci occurs less frequently

In the 8 pathological sera (3 with γ_A and 5 with γ_G paraproteinemia) with lack of antibodies against staphylococci, no detectable antibodies were found against all the other antigens tested

Isolated paraproteins

The isolated paraproteins were tested against staphylococcus extracts in order to determine if they had antibody activity It should be emphasized that the isolated paraproteins had been concentrated to the same percentage amount as in the whole sera

In the gel diffusion methods all the paraproteins (15 of the γ_G type and 1 of the γ_A type) gave rise to precipitate formation against staphylococcus antigens When compared with that present in the corresponding whole serum such positivity always appeared weaker (fig 4 A and B) Moreover, it was specifically and exclusively directed against the strains which gave a positive reaction with the serum Using the bacterial extract as antigen and the paraproteins as antibodies immuno electrophoresis revealed that the precipitation arc had the characteristic pattern of a deflecting arc (fig 4, C) No precipitate was found medially for the paraprotein arc Thus no precipitate of normal γ globulin was found

Furthermore in one pathological serum whose pure isolated paraprotein was available a positive reaction against the strain EA 1 a of pneumococcus was also detected When the isolated paraprotein consisting of only one abnormal electrophoretic peak was tested against the same strain a weaker positivity was similarly obtained All the other strains of pneumococcus and streptococcus tested against the isolated paraprotein, failed to produce a positive reaction thus suggesting that the antibody activity of the paraprotein was specifically directed against the only strains (in this case Cowan's types I and 3, and pneumococcus EA 1 a) against which antibodies had been demonstrated in whole serum

Quantitative estimations

In order to assess how much of the antibody activity demonstrated in some paraproteinemic sera was due to the corresponding paraprotein quantitative estimations were carried out in 12 cases Moreover, it was thought of interest to ascertain whether or not antibody activity of the pathological sera against Cowan's types I and 3, although of common occurrence was quantitatively comparable to that of normal controls

Of the cases investigated only I was of the γ_A type all the others being of the γ_G type The results obtained (table IV) demonstrated that the precipitating power of the isolated paraproteins against the A antigen from Cowan's type 3 is low in all the abnormal proteins tested the highest value corresponded to 8 6 % and the lowest to 1 1 % of the precipitating power of 100 μg normal γ_G globulin per 100 μl No definite relationship can be established between quantitative antibody activity and amount of anomalous protein present in each serum

In the only case of γ_A type paraprotein the precipitating power being referred to that of normal γ_G globulin is of course arbitrary However although approximate it shows that the antibody activity is

TABLE III Precipitating power (P P) *of some myeloma sera* against the 'A antigen from Cowan s type 3 staphylococcus aureus (referred to the P P of a normal pooled human serum)

No	Serum total protein (g %)	Content of normal γG globulin (g %)	Precipitating antibody concentration (mg %)	(%)
1	16 0	2 5	1 400	84 0
2	14 0	1 5	1 200	72 0
3	8 5	0 8	400	24 0
4	11 5	0 5	200	12 1
5	13 0	1 0	201	12 2
6	15 0	0 4	32	2 0
7	12 0	0 7	120	7 2
8	12 0	0 3	32	2 0
9	12 5	0 2	24	1 4
10	15 8	1 5	1 150	70 0
11	16 3	0 6	125	7 3
12	12 0	0 5	64	3 8

more or less in the same range also in this type of anomalous protein

Table III reports the precipitating power of the homologous sera from which paraproteins were isolated The values obtained, calculated as percentages of the precipitating power of a pooled normal human serum against the same *A antigen demonstrate a considerable* variation from case to case The range goes from almost normal (84 %) down to very low values (1 4 %) When the results were compared with the level of normal γ_G globulin in each serum a general correspondence was found between *antibody activity and amount of* normal γ_G globulin, indicating that the content of the normal γ_G globulin and not the quantity of the anomalous protein, must be regarded mainly as being responsible for the variable antibody activity of whole serum

The precipitating power of the isolated *paraproteins may in part* be caused by minute amounts of normal γ globulins, not traced by immuno electrophoresis However, the precipitating power is also in part caused by the paraprotein itself, because immuno electrophoresis revealed, as indicated in fig 4 C, the paraprotein precipitate developed with the bacterial extracts as antigen (vide infra)

Discussion

Detection of paraproteinemia by immuno electrophoresis has been considered for a long time as diagnostic of myelomatosis or plasmocytoma if the *anomalous protein* was of the γ_G or γ_A type, and of Waldenstrom's macroglobulinemia if it was of the γ_M type However, in the last years the *random* occurrence of paraproteinemia has been described also in other rare disorders (6, 24, 25, 43 47, 52 58, 61)

A so called 'essential paraproteinemia has been described, i e the im

muno electrophoretic detection of paraproteinemia without any clinical sign which could explain the serological abnormality (30, 43, 60) But in some of these cases also the successive appearance of the clinical features of M M has been reported (44) It should, therefore, be concluded that up to date the individuality of both atypical and essential" paraproteinemia cannot be excluded with certainty, but that in every case they must be regarded as of rare occurrence The paraproteinemic sera used in the present work are, therefore, selected on basis of the immunologic criterias of *paraproteinemia and do not exclusively* originate from M M patients

The results obtained in this study confirm and extend what has already been reported by several authors (7 18 35, 37, 38, 42, 63, 66) antibody synthesis is severely depressed in paraproteinemia, furthermore, the possibility that paraproteinemia may constitute an early humoral sign of M M would also suggest that the antibody deficiency syndrome occurs precociously, sometimes even before the disease is recognizable Although impaired, however antibody production is still similar to that of normal controls as to the frequency of occurrence of each individual antibody i e the bacterial strains which more frequently are found to produce antibody response are the same in normal controls as well as in paraproteinemic patients

An apparently different immunological responsiveness has been found with some staphylococcus antigens the occurrence of antibodies against Cowan s types 1 and 3 was in fact only slightly decreased in myeloma and other forms of paraproteinemia as compared with healthy persons, and the difference was not statistically significant However, quantitative estimations made it clear that the anti A antigen activity, although sometimes normal, exhibited fluctuation from case to case, and was evaluated electrophoretically as proportional to the level of normal γ_G globulin

The widespread occurrence of staphylococci and the strong antigenic properties of their main antigenic determinant (A antigen) would seem to indicate that a continuous stimulation of *the reticulo endothelial system (R E S)* is effective in producing an almost normal antibody synthesis in M M To our knowledge such behaviour has not been reported previously

The well documented secondary hypoimmunoglobulinemia (16, 23, 36 48) would, therefore, be regarded as being responsible for the clinical observations of recurrent infections in patients with myeloma and would find its logical explanation in the condition of immunoparesis (63) In M M and other forms of paraproteinemia the R E S being mainly engaged in producing M-component molecules, would only be able to synthesize a reduced amount of normal immunoglobulins

However, several observations suggest that the relationships between clinical features, type of anomalous protein and hypoimmunoglobulinemia have not so far, been sufficiently elucidated

1 Although a decrease of the normal immunoglobulin content is common to practically all myeloma patients it is a fact that some of them more than others

TABLE IV Precipitating power (P P) of isolated paraproteins against the 'A antigen from Cowan's type 3 staphylococcus aureus (referred to the P P of 100 μg normal-γ_G globulin per 100 μl)

No[1]	Type of the anomalous protein	Concentration of the isolated anomalous protein (g %)[2]	Precipitating power (%)
1	γ_G	6 1	0 9
2	γ_G	4 2	1 1
3	G	4 3	3 1
4	γ_G	1 9	8 6
5	γ_G	4 2	2 5
6	γ_G	7 3	1 9
7	γ_G	1 2	2 7
8	γ_G	0 7	7 2
9	γ_G	1 4	6 5
10	G	3 7	4 0
11	γ_G	2 8	2 2
12	A	3 2	2 1

[1] The paraproteins Nos 1 to 6 were isolated by column chromatography of ammoniumsulphate fractions precipitated from the 1 8 M supernatant i e above the normal precipitation range of the G globulin

[2] The protein values are based upon the estimation of tyrosine using this amino acid as standard (Lous et al 1956)

show a susceptibility to infectious diseases, often in a recurrent way which is independent of the normal γ-globulin level Also the amount of anomalous protein does not seem to be of decisive importance, as hypoimmunoglobulinemia has been demonstrated in some myeloma sera without myeloma protein (16)

2 Infections and antibody deficiency disorder are common to γ_A- as well as to γ_G myeloma Yet it has been found that normal γ_G globulins are decreased less in the first type than in the second (16)

It seems, therefore, likely that the plasma cell malignancy might determine an *impairment of the antibody* producing system through an accelerated degradation rate more than through a decreased synthesis of normal immunoglobulins (18) This has already been *demonstrated in γ_G-myeloma* (11, 39, 57), whereas in γ_A myeloma the γ globulin survival has been found to be more or less normal suggesting that in this case an impaired γ globulin formation *plays the main role* On the other hand, plasma cells producing γ_A myeloma globulins have been described as possessing histological and topographical differences from those producing γ_G-*myeloma globulins* (12, 26)

Isolated paraproteins seem to exhibit antibody specificity against some bacterial antigens The criteria of purity of the isolated paraproteins, the strict correspondence of the *antibody* behaviour between sera and paraproteins in the agar gel diffusion test, and the characteristic shape of the deflecting arc in immuno electrophoresis all constitute reliable evidence for this antibody specificity Furthermore detection in one case of pure, isolated paraprotein of antibody activity against some strains of different bacteria (in the specific case, 2 strains of staphylococci and 1 of *pneumococci*) may suggest a paraprotein to be composed of an abnormal collection of immuno-

globulins possessing polyvalent antibody activity Previous unsuccessful attempts (45) could be explained by either too low protein content in the antigen samples tested or effective failure of antibody activity against the antigens investigated

From table IV it is obvious that the precipitating power of the isolated anomalous protein is always below 8 6 per cent of maximal capacity Accepting that the precipitating power must be caused exclusively by impurities of normal γ_G globulin, the content of normal γ_G globulin in these samples should be in the range of 46 2 to 163 5 mg % (W/V)

These amounts are of a size detectable by diffusion in gel methods (46) A normal γ globulin precipitate was, however not found by these methods and the precipitation ranges of the isolated abnormal paraprotein fractions in cases 1 to 6 were above and outside the precipitation range of normal γ_G globulin These data correlated with the fact that the deflecting paraprotein arc was unveiled in immuno electrophoresis of the isolated γ fraction using bacterial extracts in the antibody trough show that the data given may give at least semiquantitative values of the antibody titer of paraprotein in the samples investigated

Quantitative estimations showed that the anti staphylococcus activity of each paraprotein was always weak when compared to that of normal human γ_G globulin The detection of antibody activity in the case of purified γ_A-myeloma protein points towards the hypothesis that in normal sera a part of anti staphylococcus activity might be associated with the γ_A globulin fraction

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1 F as to wh be regard though inc they repre thesized x malignan demonstra d a the thesis of tt ra Recent studies the antibody co plex , globulin about 1 % of its that in normal ny chemical differe molecules of γ_G g animal which ar fi n the particular configuration of the antibody combining site (see the review by Porter (49))

It would therefore seem more likely that in paraproteinemia the R E S synthesizes globulins which are presumably abnormal although closely related to the normal globulins Occasionally the synthesis of the myelomatous protein does not affect the small antibody combining site and hence globulins are formed which still keep their antibody properties In other words, antibody function and distortion of immunoglobulin structure are often but not necessarily incompatible This might account for the little activity found in the isolated paraproteins and for the absence

of a definite relationship between antibody activity and amount of M-component.

2. According to Burnet's clonal selection theory (3), M.M. and Waldenstrom's macroglobulinemia represent the result of the malignant proliferation of *one specific clone of cells*. The stimulus to the abnormal activity might be extrinsic to the cells or intrinsic, i.e. due to a somatic mutational change. In either case a specific, abnormal, individual immunoglobulin is formed. Waldenstrom has named this kind of irreversible proliferation "monoclonal gammopathy" (62).

As each clone produces one specific, antibody-type under normal conditions, its malignant development is supposed to subvert the antibody specificity. This study suggests, however, that the tumoral aberration is not of one single clone, but probably of closely related clones can be considered a more suitable hypothesis in this process disappearance of antibody *specificity should be considered of frequent* but not constant occurrence.

3. The biological role of every circulating antibody is its protective function, viz. its ability to react specifically with the corresponding antigen. Quantitative estimations of the paraprotein antibody activity and the clinical observations of the unusual susceptibility of myeloma patients to infections seem to indicate that paraproteins play an almost insignificant role in the mechanism of humoral immunity. The administration of normal, human, polyspecific gammaglobulins in these patients should, thereby, still be regarded as a useful therapeutic treatment.

Summary

93 sera with paraproteinemia (27 of the γ_A-type and 66 of the γ_G type) were investigated for the occurrence of antibodies against some strains of bacteria belonging to *Streptococcus pyogenus*, *Diplococcus pneumoniae* and *Staphylococcus aureus*.

The results obtained demonstrate that antibody synthesis is severely depressed in multiple myeloma and other forms of paraproteinemias as compared with normal controls. The impairment of the antibody producing system seems independent of the type or amount of anomalous protein, electrophoretic pattern, total protein content and age and sex *distribution. One exception was found* concerning antibodies against Cowan's types 1 and 3. In this case the almost normal findings may suggest that the extremely widespread occurrence of staphylococci on the skin and mucous membranes, and the subsequent continuous stimulation of the reticulo endothelial system are capable of producing a *practically normal* immunological response.

12 pure, isolated paraproteins (11 of the γ_G type and 1 of the γ_A type) were tested against staphylococcus antigens in order to ascertain their possible antibody activity. Positive reactions were found in all of them. In one case the simultaneous occurrence of antibody activity against one strain of pneumococcus was also demonstrated. The high degree of purity of the isolated paraproteins, the specific and exclusive reactivity against the bacterial strains which gave a positive reaction with *the whole serum* and the typical aspect of a deflecting bow in immuno electrophoresis, indicate that

paraproteins do possess antibody specificity

Quantitative estimations of the precipitating power against the A antigen from Cowan's type 3 were carried out in 12 paraproteinemic sera and in the corresponding pure, isolated paraproteins It was found that

1 In paraproteinemic sera the precipitating power ranges from almost normal to very low values, and is proportional to the amount of normal γ_G globulin present in each serum

2 The precipitating power of the myelomatous protein is always weak and is not related to the type and amount of the M component

The significance of these results is discussed In particular it is suggested that the structural abnormality of the myelomatous protein may often but not necessarily be incompatible with its antibody specificity as the small antibody combining site represents only about 1 % of the complex γ globulin molecule

Interpretation of these data with the clonal selection theory biological value of paraproteins in the humoral defence mechanism and therapeutic involvements are reviewed

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